SCIENTIFIC OPINION

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Survival, spread and establishment of the small hive beetle (*Aethina tumida*)

EFSA Panel on Animal Health and Welfare (AHAW)

Abstract

The small hive beetle (SHB) is still present in Calabria one year after its first detection in September 2014. Detailed epidemiological studies would improve our knowledge of the survival, spread and establishment of the pest. Movement of an infested hive could spread SHB rapidly over large distances. Modelling of SHB spread in absence of movement of hives, suggests that natural spread of the beetle alone will take more than hundred years to reach Abruzzo from Calabria (around 250 km). A model considering the ownership of multiple apiaries per beekeeper indicates that spread would be 10 times faster. Opportunity maps indicate that, once introduced, the SHB could complete its life cycle in all EU Member States between May and September. It is recommended that restrictions on the movement of honey bees, bumblebees and commodities from infested to non-infested areas be maintained until SHB is eradicated, to prevent spread of the pest. Strengthening visual inspection, preventing infestation using a fine mesh and issuing a health certificate for intra-EU trade of queen bees, within 24 hours before dispatch, could reduce the risk of SHB transmission via consignments. In general, visual inspection of the beehive, as described in this document, is the preferred method of detecting SHB. Traps could help to detect and reduce SHB infestation levels. Maintaining good honey house hygiene and good beekeeping practices are the most important measures to control SHB where eradication is no longer the objective, given that no approved veterinary medicine is available in the EU. A field experiment found natural infestation of commercial bumblebee (*Bombus impatiens*) colonies placed next to SHB-infested honey bee hives. However, there are no data published on SHB infestation in natural bumblebee colonies. Studies are needed of the capacity of *B. terrestris*, occurring in Europe, to act as a SHB host.

Keywords: *Aethina tumida*, small hive beetle, spread, establishment, mitigation measure, surveillance

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Summary

The European Commission requested scientific advice from the European Food Safety Authority (EFSA) on the small hive beetle (SHB, *Aethina tumida*), a bee-brood scavenger of *Apis mellifera* (honey bee), *Bombus* spp. (bumblebee) and *Melliponini* (stingless bees). In the first Term of Reference (TOR), EFSA was asked to assess the risk of survival, spread and establishment of SHB in and from Calabria and Sicily into other parts of Italy and the EU. The outbreak in Calabria and Sicily is described based on the data provided by the Italian authorities. To model the spread of SHB, two separate but similar mathematical models were constructed to simulate the dispersal from infested to non-infested apiaries. The models allow for the spread of SHB either solely due to proximity to infested apiaries (distance-only model) or due to proximity and through beekeepers infesting their other apiaries by unintentional transfer of the beetle (distance and ownership model). Movement of an infested hive would facilitate a rapid spread of SHB over large distances. Modelling SHB spread in the absence of movement of hives suggested that with natural spread alone it will take more than 100 years for the beetle to move northwards to Abruzzo from Calabria (around 250 km). A model considering the ownership of multiple apiaries per beekeeper indicates that spread would be 10 times faster. The new SHB detections in Calabria in September 2015 corroborate the modelling, which has indicated that the infestation has not been eradicated.

Opportunity maps based on calculated soil temperature at 20 cm depth indicate that, once introduced, the SHB could complete its life cycle in all the EU Member States (MS) between May and September. However, if temperatures are below 10°C, adult SHB can survive and overwinter in the honey bee colony cluster. The probability of SHB introduction from Italy to other MS is mainly dependent on the sensitivity of the test to detect SHB in traded consignments and the number of shipments arriving in a country in a given time period. Implementing sensitive SHB testing in consignments could decrease the probability of SHB introduction around 20-fold compared with absence of SHB testing. As SHB prevalence in an area depends on the control measures in place, the probability of introduction will increase when SHB prevalence increases, and could be from 2.5 up to 7 times higher if no SHB testing is in place. The lack of reliable information on the actual SHB prevalence in southern Italy, the sensitivity of the test used and number of consignments shipped between MS did not allow concrete estimations reflecting the field situation.

The second TOR requested that EFSA assess risk-mitigating factors that could potentially be effective in ensuring safe intra-EU trade of live bees, apiculture products and by-products in terms of preventing the transmission of SHB. Based on the scientific literature and qualitative evaluation by experts, detection of SHB by visual inspection and subsequent delivery of a health certificate, within 24 hours before dispatch, has been found to be highly effective and feasible for consignments of queen bees only. It is still impossible to obtain a complete dataset on European beekeeping because of the high variability of colony registration requirements in the MS. Furthermore, it is concluded that use of fine mesh with maximum 2 mm pore size to avoid contamination during transport is highly effective and feasible for consignments of bees, bee products to be used in apiculture, non-extracted comb honey and used beekeeping equipment. However, feasibility decreases as a function of the size of the consignment. Currently, implementation of precautions to prevent contamination of a shipment is required only in the case of import from third countries. For consignments of bee products to be used in apiculture, freezing and desiccation are highly effective and feasible in reducing the risk of SHB transmission. For consignments of used beekeeping equipment, freezing, heating and desiccation are highly effective in reducing the risk of SHB transmission. The feasibility depends on the size of the shipment and on facilities available. The assessment assumed that visual inspection is perfectly implemented, although this might not always be the case in practice. Therefore, it is recommended that the SHB status of the area of origin of consignments be taken into consideration when issuing health certificates and that visual inspection of bee consignments undergoing intra-EU movement be strengthened, as it is already done in the case of import from third countries. The availability of a register of the location of apiaries and number of hives within an area together with tracking information on the travel route of shipments would be essential to facilitate epidemiological investigations in the event of an outbreak. Moreover, even in the absence of a national registration system, it is recommended that beekeepers keep records of their bee movements to facilitate investigation of outbreaks. Finally, making more use of fine mesh (with maximum 2 mm pore size) for intra-EU trade of commodities could reduce the risk of SHB transmission via consignments.

The third TOR requested EFSA to assess the risk mitigating factors and methods in apiaries, alternative to currently employed complete destruction of the apiary and additional risk mitigating factors that may be applied in controlled environments for queen producing. Based on the scientific literature and experience of outbreaks, it was concluded that visual inspection is the most frequently used method to detect SHB in apiaries and, depending on the expertise of the inspector and the rigour of the inspection process, it can identify not only the pest in its different life stages, but also damage caused by the pest. Traps and polymerase chain reaction (PCR) analysis of hive debris are other methods that can be used in apiaries in addition to visual inspection, although the PCR method needs to be validated in field conditions to better evaluate its performance. Good honey house hygiene and good beekeeping practices are the most important measures to control SHB in an infested area where eradication is no longer the objective, given that no approved veterinary medicine is available in the EU. Based on experience in Australia, Canada and the USA, traps could be used to reduce the SHB population in infested areas. No specific control measures to keep honey bee queen production free from SHB in an infested area are available, and there is no EU legislation in place regarding movement control of honey bees, bumblebees or commodities in a SHB-infested area. Soil treatment with pyrethroids to control SHB should be applied only in the event of comb damage, and exposure of non-target species to pyrethroids should be avoided. It is also recommended that restrictions on the movement of honey bees, bumblebees and commodities from infested to non-infested areas be maintained until SHB is eradicated in order to prevent spread of the pest.

The fourth TOR requested EFSA to review the surveillance in assessing an area's freedom from SHB, including the size (radius of) of the areas to be surveyed, in order to provide solid bases for regionalisation policy. According to modelling that took into account inspection and mitigation measures implemented in Italy, including establishing a protection zone of 20 km radius, reducing the surveillance zone radius from 100 km to 50 km will at least double the probability of SHB escaping from that surveillance zone, from 0.025 to 0.05. The World Organisation for Animal Health (OIE) requirement to implement a 5-year monitoring programme to substantiate SHB freedom is based on the current knowledge of the biological characteristics of the pest. However, any recommendation on the duration of such a monitoring programme is subject to high uncertainty because relatively few data are available. Passive surveillance is implemented in all MS as SHB detection is notifiable. Guidelines on surveillance strategies have been published by the European Reference Laboratory on Honey Bee Health. Training of beekeepers and veterinary inspectors is recommended as it will facilitate rapid SHB detection.

The fifth TOR requested EFSA to assess the susceptibility of kept bumblebees (*Bombus terrestris*) to SHB or their capability to spread SHB. A field experiment found natural infestation of commercial bumblebee *B. impatiens* colonies placed next to SHB-infested honey beehives. However, there are no published data on SHB infestation in natural bumblebee colonies. Food resources and conditions in bumblebee colonies are attractive to SHB and suitable for its development. Therefore, the possibility that bumblebee colonies act as a reservoir for SHB cannot be excluded. Studies of the capacity of *B. terrestris* (occurring in Europe) to act as an SHB host, are needed since data are currently available only for *B. impatiens*. Furthermore, kept bumblebee boxes should be destroyed after the pollination service.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The recent EFSA opinion on small hive beetle (SHB, *Aethina tumida*) and *Tropilaelaps*[1] has addressed comprehensively the risk of entry of these pests into the European Union (EU). Since the publication of the opinion in early September 2014, SHB has been detected in Calabria, Italy, with dozens of infected apiaries within an area of 20 km radius. Surveillance outside this area but within and beyond a radius of 100 km has not detected other occurrences. However, the tracing of colonies practicing transhumance, within the area and having left the area, were later confirmed infected, leading to the discovery of SHB in Sicily in early November 2014.

These areas are a major source of queen bees produced in large quantities for shipment to many places in the EU, as well as for mobile apiaries or transhumant hives moving in from elsewhere and leaving afterwards for 3 flowering seasons from spring to late autumn.

Italy has implemented regional and national measures to contain, survey and if possible to eradicate SHB.[2] This involves destruction of infected apiaries and restriction of movement of colonies and certain apiculture products, by-products and beekeeping equipment. A Commission Decision has also been adopted covering aspects related to intra-EU trade[3]. In particular colonies and queens must not leave restricted areas. However, it should be noted that anecdotal evidence suggests that intra-EU movements of live bees are liable to illegal activities, which are difficult to control, particularly in the case of queen bees that can be easily hidden or sent (e.g. by post). This exposes the rest of the EU to a risk of introduction of the SHB, despite sound rules, especially if those are perceived unnecessarily restrictive.

While the current aim of the Italian veterinary services is to eradicate the SHB, it is uncertain whether this is possible and if not which are the best method to mitigate against its spread as well as the damage caused in apiaries. It is also unclear whether SHB is capable of surviving various European winter conditions, to spread and to establish permanently either in the already infected areas or beyond or to become endemic. There are uncertainties as to whether it would have a major impact on the bee population and on the beekeeping activities implying serious socio-economic consequences for the beekeeping sector that are disputed at least by some, e.g. by a certain Italian beekeepers' organisation.

In North America, the introduction of the SHB caused damages to the beekeeping sector, mainly in the southern States of the USA, while in the northern States damage was more limited and survival of SHB is less clear. In Canada its survival, spread and damage remained low, raising the question of its ability to become established.

Very few animal health requirements for SHB in the usual intra-EU trade context have been established, based on the fact that SHB has been hitherto exotic in the EU. The relevant Directive 92/65/EEC[4] lays down animal health requirements for intra EU movements of bees and the model health certificate for such movements. It should be noted that these requirements are simply meant to create in an initial phase an automatic block for movements of bees in case an outbreak would be notified in a Member State. They are not suitable to handle trade between infected areas and free areas.

In order to avoid the introduction into the EU of the SHB with imports of live bees, Regulation (EU) No 206/2010[5] contains the requirements and the model certificate for import of live queen bees and queen bumblebees. These requirements have been assessed favourably by the previous EFSA opinion. Nevertheless these requirements still stipulate freedom from SHB within an area of 100 km radius. This is a condition that large parts of Italy are unlikely to be able to fulfil, should similar rules apply to them as to third countries, unless SHB is completely eradicated.

[1] http://www.efsa.europa.eu/en/search/doc/3128.pdf
[2] http://www.izsvenezie.com/aethina-tumida-in-italy/
[3] doc [SANCO/7095/2014].
[4] OJ L 268,14.9.1992, p. 54.
[5] OJ L 73, 20.3.2010, p. i.

In order to support the Commission and the Member States in improving the control, eradication and trade measures as regards the SHB, scientific advice from EFSA is required in this area. The Commission therefore considers opportune to request EFSA to assess all the available scientific information and to evaluate the risk of survival, establishment and spread of the SHB in the EU.

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide a scientific opinion on:

1) the risk of survival, spread and establishment of SHB in and from Calabria and Sicily into other parts of Italy and the EU under various scenarios:
    a) by natural movements of live bees (*Apis mellifera*), including feral colonies and of the SHB, under currently applicable emergency conditions, taking into account especially relevant geographical and meteorological conditions;
    b) by natural movements of live bees and of the SHB and by intra-EU movement of bee colonies, queens and apiculture products and by-products from infected areas, under identified risk mitigation measures;
    c) by natural movements of live bees and of the SHB and by intra-EU movement of bee colonies, queens and other products and by-products in absence of EU rules (i.e. similar as applicable to *Varroa* mites)
2) risk mitigating factors that could potentially be effective in ensuring safe intra-EU trade of live bees (both colonies and queens) and apiculture products and by products as regards the transmission of SHB;
3) risk mitigating factors and methods in apiaries, including quick diagnosis and potential treatment(s), alternative to currently employed complete destruction of the apiary and additional risk mitigating factors that may be applied in controlled environments for queen producing;
4) surveillance (active and passive) in assessing freedom of areas from SHB including the size (radius of) of the areas to be surveyed in order to provide solid bases for regionalisation policy;
5) susceptibility of kept bumblebees (*Bombus terrestris*) to SHB or their capability to spread SHB as vectors.

1.2. Interpretation of the Terms of Reference

According to a previous EFSA opinion (EFSA AHAW Panel, 2013), '*The SHB is a bee-brood scavenger of Apis mellifera (honey bee), Bombus spp. (bumblebee) and* Melliponini *(stingless bees). Mature larvae leave the hive and burrow in soil to pupate. This coleopteran is a flying, free-living predator that can survive and reproduce on a variety of ripe fruits, but not on vegetables, plants or flowers. Adult SHB can detect airborne volatiles produced by A. mellifera and Bombus spp. and thereby can be attracted to the odour of bees and bee products that have come into contact with bees. The pest is native to Africa but has spread to North America and Australia during the past 20 years. The larval stage of the pest is destructive to a bee population, whereas the adults have little impact. The larvae burrow through combs, eat honey and pollen, kill bee brood and defecate in honey, which subsequently ferments*.' The interested reader could consult this document for more background information on the pest and/or a recently published review paper (e.g. Cuthbertson et al., 2013).

TOR1a provides a descriptive analysis of the SHB outbreak in Calabria and Sicily. Based on outbreak data provided by the Italian authorities, survival and spread of SHB via natural movement of the pest within Calabria and Sicily and to other parts of Italy has been simulated using mathematical modelling taking the applied emergency conditions[6] into account. In addition, this TOR aimed to identify natural barriers that might have contributed to the limited spread of SHB, and areas within Europe where SHB

[6] No movement of bees is allowed (i) within the protection zone (20 km radius around the first detected infested apiaries) and (ii) from the protection/surveillance zone to SHB-free zones within Italy and (iii) from the SHB-free zones to the protection/surveillance zone. Movements within the surveillance zone (100 km radius around the protection zone) are allowed following two apiary inspections with negative result 21 days apart. However, colonies in the protection zone are split, to reduce natural swarming as much as possible. This is a derogation to the current restriction on the movement of colonies within the protection zone since split colonies have to be separated by at least 3 km (Note of the Italian Ministry of Health n. 0010658-23/04/2015-DGSAF-COD_UO-P). Most of artificial swarms were produced between April and June 2015.

is not likely to become established. However, the coarse spatial resolution of the datasets did not allow robust inferences regarding natural barriers to be drawn. In TOR1b, the likelihood of SHB introduction into other MS via intra-EU movement of consignments (either bees or non-living material) from an infested area was calculated for different scenarios by changing test sensitivity, SHB prevalence at the place of origin and number of shipments. Scenarios taking into account a lower probability of detecting a shipment infested with SHB (i.e. lower prevalence of SHB in the shipment as a result of measures aimed at eradicating or drastically controlling SHB infestation) and higher test sensitivity are probably representative of the situation in which EU rules are in place. The reverse could be representative of the situation in the absence of EU rules (TOR1c). More detailed data on the actual SHB prevalence in the area of origin, the sensitivities of the test used and number of consignments shipped between MS would be required to provide estimates that reflect the real situation.

An assessment and providing practical guidance on 'monitoring SHB freedom of a consignment', 'isolating the consignment' and 'treating to prevent SHB infestation in a consignment' has been done in TOR2, since these risk mitigation measures were identified as being highly effective and feasible in reducing the risk of SHB transmission for consignments of live bees or non-living bee products and materials (see EFSA, 2015). Only treatment methods that keep the consignment intact are included. Measures that are considered highly effective and feasible could be applied to ensure safe intra-EU trade. Surveillance as such is not described since the focus of this TOR is at the consignment level, although release of a health certificate indicating the SHB status of the zone will require some surveillance (see EU Reference Laboratory for Honeybee Health guidelines; Chauzat et al., 2015).

To address TOR3, risk mitigation factors that could be applied to manage SHB infestations in apiaries located in an infested area where no eradication is undertaken are assessed. Some measures, such as monitoring SHB presence, can be used for consignments (TOR2) and in apiaries (TOR3), although their implementation might be different in each case. The implementation of mitigation measures in queen-producing facilities is also assessed. The aim of this section was to provide information that is more detailed and practical than that included in the recently published EFSA scientific report (EFSA, 2015) and which is applicable to an SHB-infested area where eradication is no longer the objective.

A model-based assessment of the effect of different surveillance radii, considering analytical and simulation approaches, has been performed in TOR4 to describe the probability that SHB escapes from the surveillance zone. Criteria for declaring SHB freedom in a previously infested area are identified and surveillance in SHB-free areas is briefly described.

The characteristics of a host species for SHB are described and the scientific evidence regarding the capacity of managed bumblebees to act as host species for SHB are assessed to address TOR5. Feral bumblebees have not been considered in this assessment.

2. Data and Methodologies

2.1. Data and approach of SHB opportunity maps

Analysis of the available scientific literature together with expertise available in the working group revealed that soil type, soil moisture and soil temperature are the main parameters determining SHB survival and establishment. A temperature below –1°C at 20 cm depth in the soil for 1 hour is considered to kill SHB pupae and prevents completion of the life cycle. Daily mean air temperatures (1 January 2014 to 31 December 2014) are available for Europe[7] for around 20 000 25 × 25 km grids. The daily mean air temperature for the period considered was transformed into soil temperature (for 20 cm depth) using a linear multiple regression model (described in Appendix A). Based on the estimated soil temperatures obtained from the model, maps identifying regions in Europe where the soil temperature is below –1°C in the different months were constructed (opportunity maps). Different colour shadings indicate the number of days in a month satisfying the condition (darker colour indicates more days). No thresholds for the survival of SHB could be defined according to soil type (data identifying in which soil types SHB (or SHB life cycle phase) would not survive are not available)

[7] Temperature data were provided to EFSA by the JRC Monitoring Agricultural Resources (MARS) unit Meteorological Data Base (EC/JRC) (http://marswiki.jrc.ec.europa.eu/agri4castwiki/index.php/Meteorological_data_from_ground_stations – last accessed 21 July 2015).

and soil moisture (pupation requires at least 5% soil relative humidity, which is present everywhere in Europe). These variables, therefore, have been not considered in the final model. The results are presented in Section 3.2.1.

2.2. Data and approach of modelling SHB spread within Italy

The outbreak has been analysed using generic approaches, since the lack of detailed epidemiological data hampered the implementation of other methodologies. A model was developed to describe the spread of SHB, which takes the distance of natural SHB spread into account (independent of whether spread is due to SHB flying alone or together with feral or managed bees). It has been validated using Italian outbreak investigation data collected between September 2014 and September 2015, although data from Sicily only cover the period September 2014 till June 2015 (timing of SHB inspection, location of apiary, positive or negative outcome from inspection; provided by the Istituto Zooprofilattico Sperimentale delle Venezie, Italy) and locations of all registered apiaries in the south of Italy (Calabria, Apulia, Molise, Campania, Basilicata and Sicily; provided by the Italian Ministry of Health). More information is provided in Appendix B. The Italian authorities are implementing a new database that will include more detailed data on apiaries, but at the time of preparing this scientific opinion this database was not sufficiently advanced to be used.

The model is used to simulate the possible spread of SHB from the south of Italy to the rest of the country, based on the available outbreak investigation data (see Section 3.1.2). In addition, the model is used to assess the impact of changing the radius of a surveillance zone on the possible spread of SHB (see Section 3.5.1).

2.3. Data and approach of estimating SHB spread from Italy to other Member States

A quantitative approach to estimate the likelihood of introduction of SHB into an SHB-free country or area through the movement of bees and bee products is essential to assess the risk of introduction. Only limited data on the trade of bees between MS are available, and numbers are not consistent between different data sources (e.g. data in the TRAde Control and Expert System (TRACES)[8] versus data published by MS organisations; see Appendix C and website National Bee Unit[9]). No data on the trade of bee products to be used in apiculture or used beekeeping equipment between EU Member States are available. In addition, the number of apiaries and their location is not available for all MS. Therefore, the likelihood of SHB spread from Italy to another MS has been calculated based on binomial principles, i.e. based on the probability of detecting a shipment infested with SHB, the number of shipments (shipment size) and the true SHB prevalence in the shipped matrix at the place of origin (see Appendix D). Some scenarios are described in Section 3.2. The lack of data does not allow the effect of presence or absence of EU rules to be addressed directly. However, scenarios taking into account different magnitudes of the probability of detecting a shipment infested with SHB (i.e. reflecting different SHB testing strategies to detect SHB in some matrices or the absence of testing strategies) were considered.

2.4. Approach of assessing risk mitigation measures

The risk mitigation measures that could be applied to effectively reduce the probability of survival, spread or establishment of SHB via consignments (identified in EFSA, 2015) are described and are based on the available scientific literature and with indication of knowledge gaps where possible (see Section 3.3). Further assessment has been done by the working group experts, by scoring for effectiveness and indicating the feasibility of the measure and level of uncertainty of the given scores (see Appendix E). When scoring the effectiveness, it was assumed that the risk mitigation measure was implemented in an optimal manner. The experts considered the worst-case scenario, in other words trade from an SHB-infested area to an SHB-free area, during the assessment. Discussion took place amongst the experts to obtain a consensus score. The rationales used as basis for the scores given are described in Section 3.3.

[8] TRACES is a trans-European network for veterinary health which notifies, certifies and monitors imports, exports and trade in animals and animal products (http://ec.europa.eu/food/animal/diseases/traces/index_en.htm).

[9] http://www.nationalbeeunit.com/public/BeeDiseases/euImportReport.cfm?year=2014 (last accessed 14 September 2015).

The risk mitigation measures that could be applied to manage SHB infestations in apiaries located in an infested area where no eradication programme is in place are described based on the available scientific literature, with an indication of knowledge gaps where possible (see Section 3.4). An overview of routine SHB monitoring and management measures in an apiary is provided.

2.5. Approach of assessing criteria for regaining SHB-free status

The modelling approach developed by Schley et al. (2009) was used to assess the size of the area that needs to be surveyed. The dispersal kernel (i.e. the probability that SHB will spread from an infested hive to a non-infested hive a given distance away) and the reproduction number (i.e. the average number of newly SHB-infested hives arising from a single infested hive) are used to calculate the probability that SHB will escape from a circular zone of a specified radius. These analytical results were compared with simulations of the SHB spread model.

The criteria described in the OIE Animal Health Terrestrial Code (OIE, 2015) are reviewed based on the current knowledge of SHB and available methodologies to assess freedom from disease (see Section 3.5.1).

2.6. Approach of assessing role of bumble bees to act as SHB host

The characteristics to be fulfilled by a species to act as a host for SHB are described. An assessment whether bumble bees fulfil these characteristics has been done based on the available scientific literature and with indication of knowledge gaps where possible (see Section 3.6).

3. Assessment

3.1. SHB spread in Italy

3.1.1. Descriptive analysis of the outbreak in Calabria and Sicily

SHB was first detected (by visual inspection) and then confirmed by morphological examination at an apiary in Calabria, a region in southern Italy with coastline onto the Mediterranean, on 5 and 12 September 2014, respectively. Following its detection, further inspections revealed a total of 60 infested apiaries (59 in Calabria and one on the island of Sicily, the latter likely due to transhumance between both regions) (see Figure 1). Wherever SHB was detected, all colonies (whether infested or not) within the apiary were destroyed by burning the combs and boxes. Of the 59 positive apiaries in Calabria, 35 were linked by ownership, i.e. they were owned by an individual with at least one other infested apiary.

No further infestations were identified between December 2014 and 24 June 2015, with 6 284 inspections carried out in total at 2 179 apiaries in Calabria and Sicily (with a maximum of 18 visits per apiary). However, a new infested temporary apiary[10] was confirmed on 16 September 2015 in the municipality of Taurianova in the province of Reggio Calabria. Both SHB adults and larvae were detected. The apiary was composed of 32 swarms, of which 20 had been produced in the municipality of Laureana di Borrello and 10 in the municipality of San Pietro di Caridà (both municipalities within the protection zone) on 6 August 2015 and had been moved for raspberry pollination to the new destination on the same date. Two natural swarms were captured near the infested apiary on 16 and 22 August and added to the apiary. These two apiaries were investigated and found negative. In the 2 weeks following the new detection, three more apiaries have been found infested by SHB within a radius of 5 km of the apiary confirmed on 16 September, and further detections occurred in October 2015. Events occurring after 30 September 2015 are not modelled in this opinion.

The affected area in Calabria has many citrus and kiwi orchards and, hence, is of interest for pollination and for beekeepers that produce honey. Movement of hives to this region occurs during the citrus-blooming period in April–May. Hives are then moved to adjacent territories in Calabria to pollinate eucalyptus in May and chestnut in May–June and, finally, in September are moved north of the infested area for the second eucalyptus blooming. After the blooming season, the hives are returned to their area of origin (the same area of Calabria, Sicily, Abruzzo, etc.).

[10] A temporary apiary composed of swarms was created for pollination purposes.

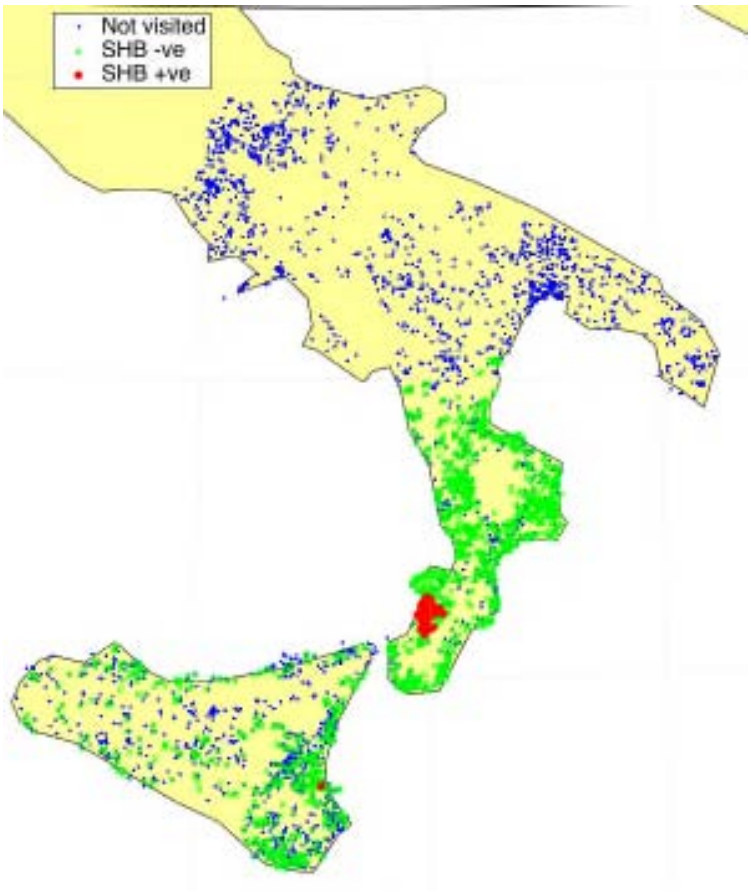


Figure 1: Spatial location of apiaries in the south of Italy with indication if SHB detection was positive or negative in the apiaries that were inspected between 5 September 2014 and 30 September 2015

The SHB-positive apiaries in Calabria are clustered, with a maximum distance between infested apiaries of 28 km. This clustering could be the consequence of geographical features (there is coastline to the west of the infested area and a mountainous regions to the south, east and partially to the north), ownership network or other factors including, but not limited to, temperature, wind, soil and apiary density that could have influenced SHB dispersal. Although data are available for some of these factors, the spatial resolution of the datasets is too coarse to allow robust inferences to be drawn. Further analysis of the outbreak (see Section 3.1.2) has been done using a MCMC scheme and a stochastic SIR model (see Appendix B), taking geographical locations and ownership details into account. The implementation of other methodologies was hampered by a lack of detailed epidemiological data, such as a systematic analysis of all apiaries around infested ones over time, tracking of data on movements of bees, bee products or used beekeeping equipment, description of environmental conditions and presence of potential reservoirs such as feral bees and bumblebees.

3.1.2. Modelling the spread of SHB

To model the spread of SHB, two separate but similar mathematical models were constructed to simulate the dispersal from infested to non-infested apiaries. The models allow for SHB spread either solely as a result of proximity to infested apiaries (distance-only model) or due to proximity and through beekeepers infesting their other apiaries through 'unintentional transfer'[11] of the beetle (distance and ownership model). Dispersal routes other than proximity are henceforth collectively

[11] This is mainly related to beekeeper actions. Unintended bee/beetle transport in a vehicle or a non-bee consignment, and perhaps not even involving the beekeepers themselves, cannot be excluded but is considered less likely.

referred to as the 'ownership network'[12]. The models were parameterised using methods which allow patterns of infestation and spread to be inferred from inspection data (see Appendix B for details).

The assumptions of the models are discussed in detail in Appendix B, but the salient points are:

- The movement of apiaries (e.g. for pollination of crops) prior to discovery of SHB is not modelled. If SHB were to spread outside Calabria and Sicily to a region without movement restrictions in place, movement of apiaries would be a major route of SHB dispersal. Also, unintended transport of beetles in non-bee-related consignments are not modelled here.
- SHB is simulated to spread directly from apiary to apiary, with a maximum dispersal distance of 30 km (estimated using empirical data of flying distances). However, it is still unclear if SHB readily travel longer distances and switch between apiaries (Spiewok et al., 2008).
- Infested apiaries are assumed to be detected immediately following infection; the probability of detection is a parameter within the models, and is determined as part of the statistical fitting scheme using the positive and negative inspection data. Owing to the absence of SHB detection with repeated visits in the available dataset (September 2014 to September 2015), attempts to increase the detection probability over time from 0 to 1 resulted in no time dependence, so detection is simply assumed to remain fixed over time.

The results of the statistical analysis show the ownership network to be pivotal in the spread of SHB. Hence, a major step towards limiting dispersal of SHB would be to take care when transporting bees, bee products intended for use in apiaries and/or beekeeping equipment between owned apiaries, so that SHB is not unintentionally introduced to multiple apiaries. However, being in close proximity certainly increases the risk of infestation (probably as a result of SHB movements), as SHB-positive apiaries in Calabria are clustered together.

The results from the models imply that the outbreak may have not been eradicated yet. In the 'distance-only' model, SHB was eradicated in 20.0% (95% credible interval (CI) 17.6–22.4%) of the outbreaks simulated up to 30 September 2015, while in the 'distance and ownership' model it was eradicated in 18.7% (95% CI 16.3–21.1%). These results are corroborated by SHB-positive apiaries being found from mid-September to November 2015. It is, of course, difficult to confirm eradication of any ongoing outbreak, and continued inspection and control efforts are required to make sure that SHB does not spread beyond the borders of Calabria into neighbouring regions such as Basilicata and Apulia.

3.1.3. Simulation of the time required for SHB to spread from Calabria to other parts of Italy

Both models were used to simulate the time required for SHB to spread northwards from Calabria to Abruzzo (around 250 km). Neither model includes spread of SHB via the movement of bees, which has not been allowed in Calabria and Sicily since September 2014 until at least the end of September 2015. In fact, current Italian (Order of the President of Calabria region no 94 of 19.09.2014) and EU (Implementing Commission Decision 2015/1943/EU) regulations ban the dispatch of consignments of bees and other beekeeping commodities from the two regions concerned to other areas of the EU. Accordingly, it is important to note that the times estimated are likely to be substantially longer than would be the case if bees were to be moved freely (or illegally). In this latter case, spread could be very rapid indeed, though a lack of data precludes quantification of how rapidly this could occur. SHB spread rapidly in the USA and were present in 19 states within three years of the initial discovery in 1998 in four Southern states: Florida, North and South Carolina and Georgia (Hood, 2000; Neumann and Elzen, 2004). This rapid spread of over 1,500 to 2,000 miles can only be explained by the movement of SHB-infested hives by migratory beekeepers for pollination or honey production purposes (Pettis et al., 2014). In Australia, SHB spread rapidly over long distances helped by the transportation of hives. For example, hives were moved from the Richmond area in New South Wales to Nambour Queensland, over 1,000 km away not long after SHB were first identified in hives in Richmond. Within two months SHB were found in hives in the Nambour area (Diana Leemon, Department of Agriculture and Fisheries, Australia, personal communication, 17 November 2015).

[12] Referring to a higher risk of an apiary being infested if the owner has another apiary that is infested. However, no statements can be made about the mechanisms underlying this higher risk.

Assuming that no movement of bees takes place, the 'distance and ownership' model suggested that it would take, on average, 22.7 years (95% credible interval CI 22.3–23.1 years) for SHB to reach Abruzzo. This reflects a rate of spread that is almost 10 times faster than that predicted by the distance-only model (average time 202 years; 95% CI 198–207 years) (see Figure 19, Appendix B). These results suggest that, in the absence of movement of honey bee colonies, the spread of SHB is very slow. Moreover, they again demonstrate the importance of ownership networks in the spread of SHB.

The 'distance-only' model was also used to simulate the time required for SHB to spread from Calabria to the border of Italy with other MS (see Figures 20 and 21 in Appendix B). These modelling results again confirm the slow spread of SHB when considering spread without movement of bees or spread within ownership networks. A lack of data on ownership networks in regions north of Molise prevented Italy-wide simulations using the 'distance and ownership' model, and the absence of data on seasonal colony movements meant that the effect of transhumance could not be evaluated.

3.2. Spread of SHB from Italy to other Member States

3.2.1. SHB opportunity maps

Environmental temperature is an important factor that affects the SHB life cycle. Development of any life stage is reported to stop below 10°C (Meikle and Patt, 2011; Bernier et al., 2014). Temperatures exceeding 35°C cause high mortality of all SHB life stages (Meikle and Patt, 2011) and experiments suggest that all SHB life stages are killed when exposed to temperatures of ≤ –1°C for 1 hour (Stedman, 2006). The soil moisture should be above 5% for pupation of SHB (Somerville, 2003; Stedman, 2006), which is generally the case in most European soils, for the majority of the year. In exceptional circumstances (long draught), coarse texture soils can record moisture below these values until around 10 cm depth. Pupation can occur in any soil type (Ellis, 2004a; de Guzman et al., 2009) although the nature of the soil type can influence pupation rates (Schmolke, 1974; Pettis and Shimanuki, 2000; Wenning, 2001). Therefore, exposure of SHB to a temperature below –1°C is considered the only available and relevant parameter in the European context that can be used to generate opportunity maps. A conservative approach was used considering that SHB cannot survive when the average mean daily soil temperature at 20 cm depth is below –1°C for at least 1 day in a month, since the majority of SHB pupae are found in the first 20 cm of the soil (Pettis and Shimanuki, 2000). Three formulas have been generated to transform air temperature (2014 data) into soil temperature at 20 cm depth, assuming that the soil was covered by grassland, arable soil or crops (see detailed description in Appendix A). Figures 2, 3 and 4 suggest that, once introduced, SHB can complete its life cycle in all MS between May and September. This seems to be independent of the soil coverage, since the figures are similar in areas of Europe that are covered with grassland, crops or orchards. However, these results do not represent the mean long-term conditions as only temperature data from 2014 are used. Furthermore, it is important to highlight that the adult SHB can survive winter inside the hive, taking advantage of the warmth and food within the honey bee colony cluster (Hood, 2000; Neumann and Elzen, 2004). Indeed, SHB readily survives in areas of North America with a colder climate, such as Minnesota and Wisconsin in the USA; SHB has also reached Canada (Dixon and Lafrenière, 2002).

A limitation of the applied approach is that snow cover effects on soil temperature are not considered in this calculation. In the case of snow cover, soil temperatures are strongly damped, and can reach close to 0°C at a depth of 0–20 cm, even under strong frost conditions (see also Appendix A). In regions with high winter precipitation and regular snow cover, this may lead to some deviation in some years regarding the freezing depth. The conservative value of –1°C as the applied limit counteracts this effect to a certain degree, but a further analysis in comparison with snow cover models should be carried out for a better quantitative estimate of potential regional deviations.

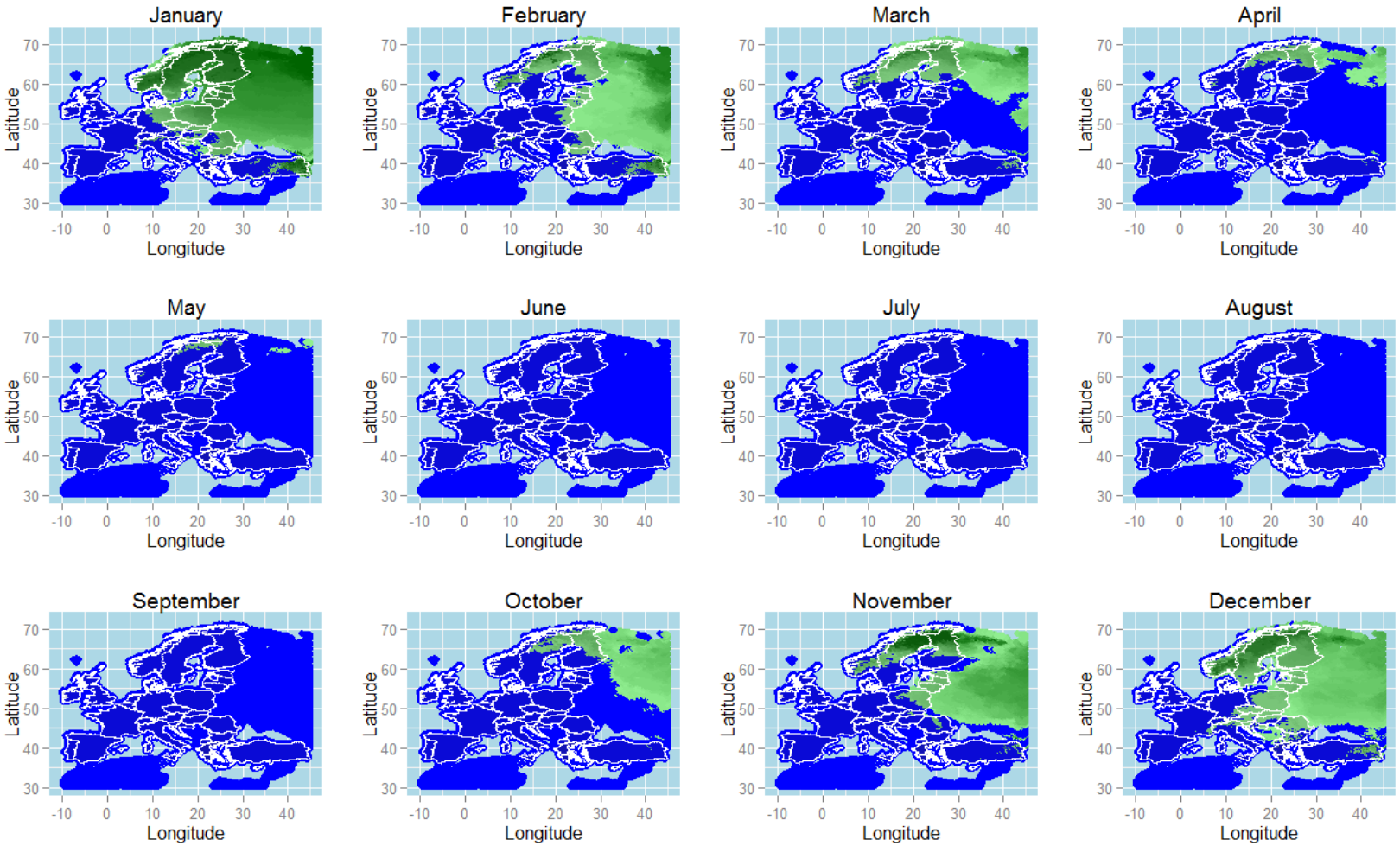


Figure 2: Maps indicating, in green, regions in Europe where the estimated maximum temperature 20 cm below ground was below –1°C on at least 1 day in the month and, in blue, regions where was not the case (assuming grassland cover for all areas of Europe and no snow cover effects)

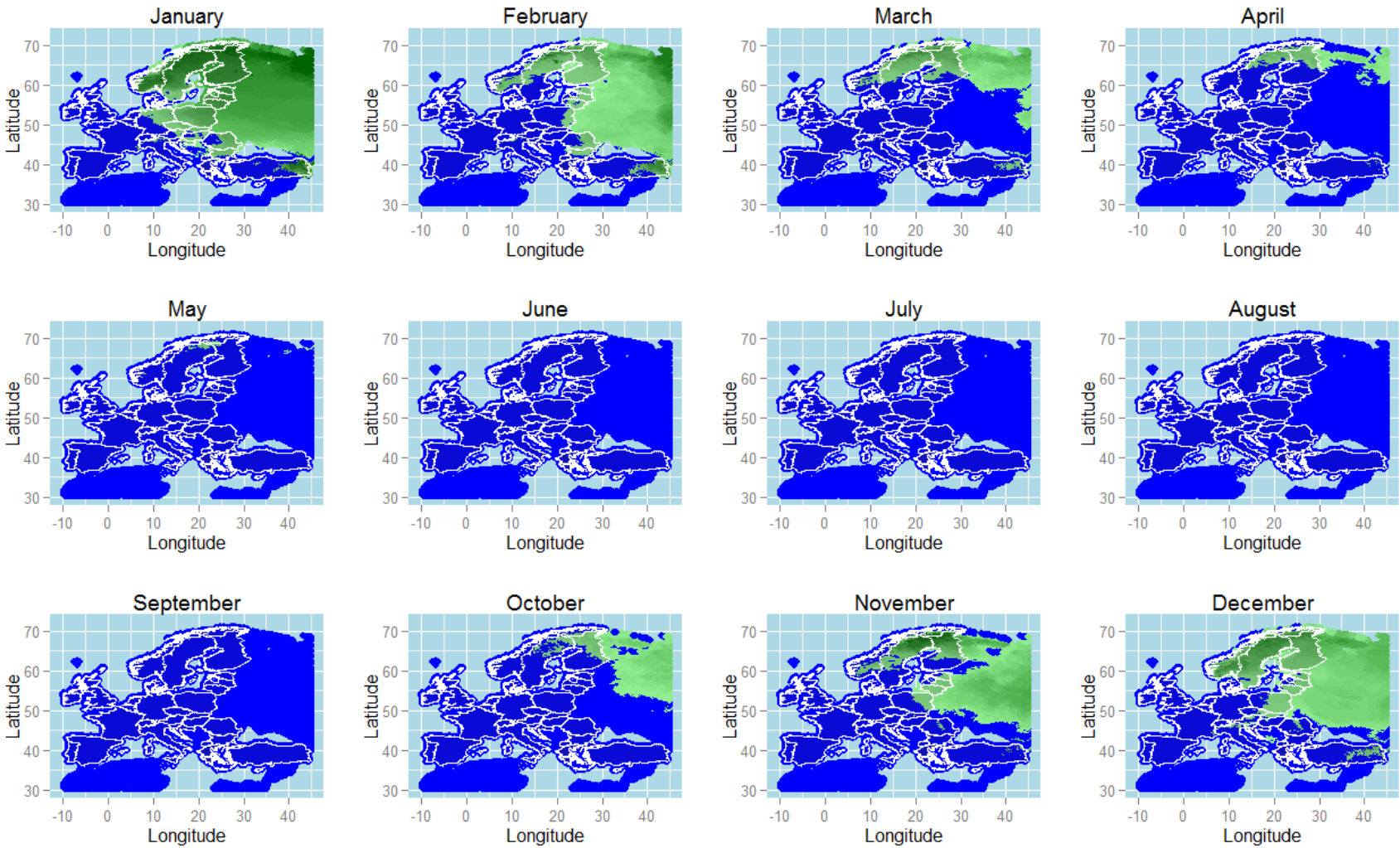


Figure 3: Maps indicating, in green, regions in Europe where the estimated maximum temperature 20 cm below ground was below –1°C on at least 1 day in the month and, in blue, the regions where was not the case (assumed that Europe was covered by crops and without any snow cover effects)

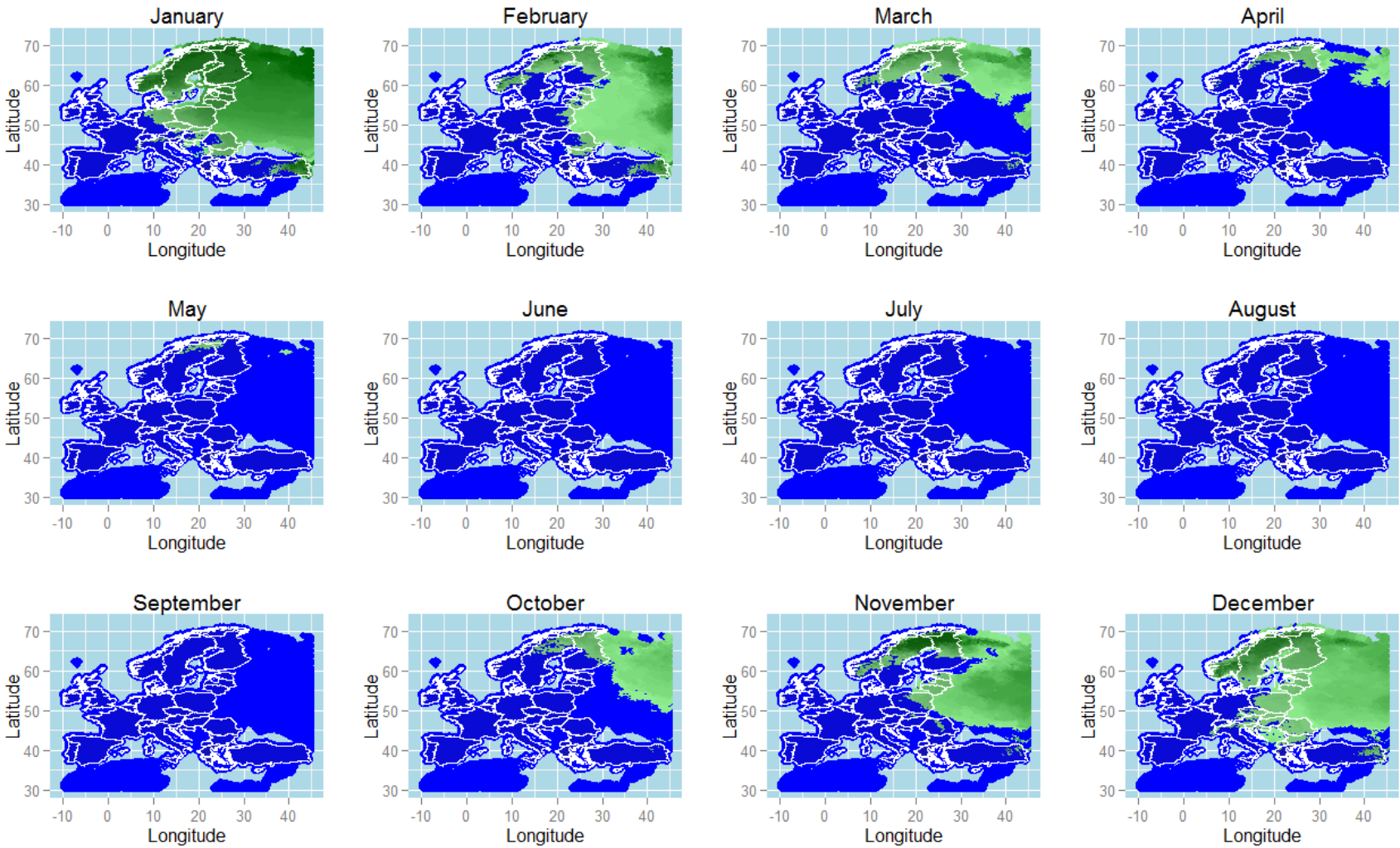
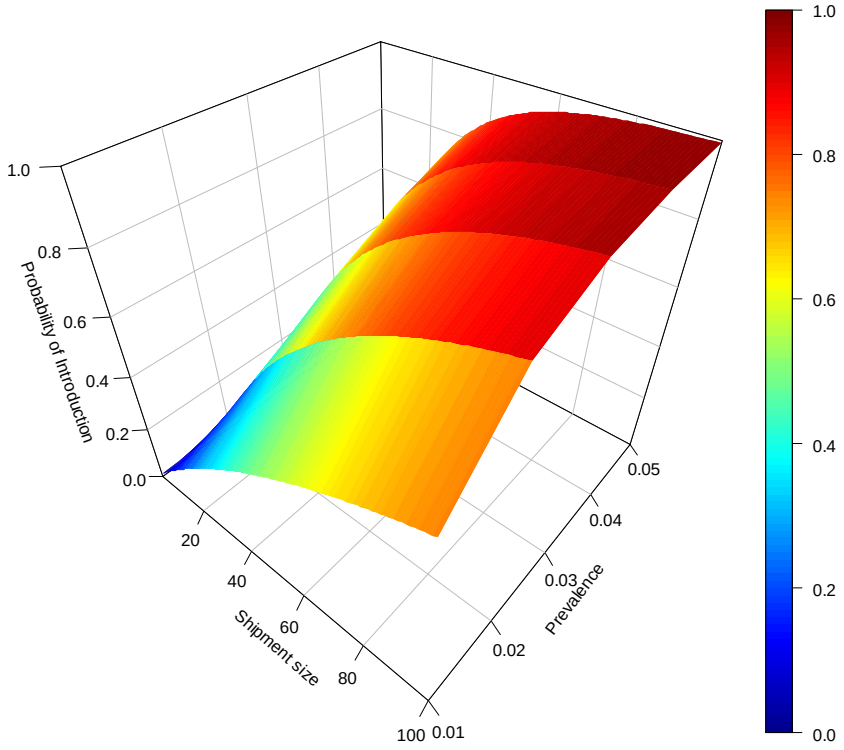


Figure 4: Maps indicating, in green, regions in Europe where the estimated maximum temperature 20 cm below ground was below –1°C on at least 1 day in the month and, in blue, the regions were was not the case (assuming that Europe was covered by orchards and without any snow cover effects)

3.2.2. SHB spread via consignments

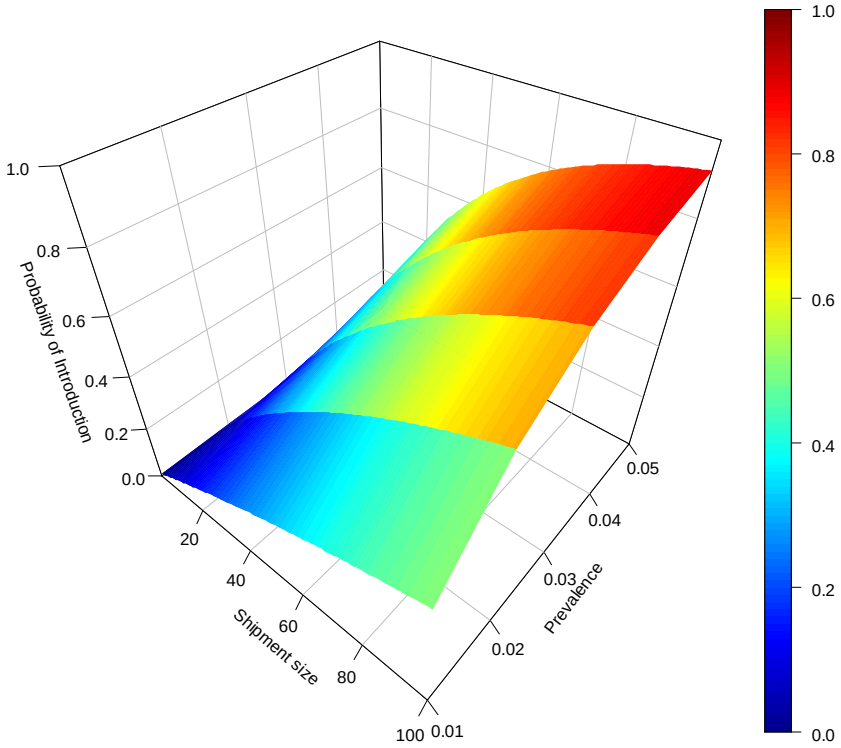
The likelihood of SHB introduction into other MS via intra-EU movement of consignments (either bees or non-living material) from an infested area was calculated and is shown in Figure 5 (see Section 2.3 and Appendix D for more details). The model considers the scenario in the absence of SHB testing (which can be described as test sensitivity equal to zero; referring to intra-EU movement of package bees[13] or absence of surveillance at the borders; see also EFSA, 2015) and when the SHB prevalence in the shipped matrix at the place of origin is 0.05 (i.e. 5%)[14]; in this case the probability of introducing SHB into an SHB-free area is approximately 1 (i.e. 100%) if the number of bee packages moved is above 100 (Figure 5A). If queens are moved (referred to as test sensitivity equal to 0.95, assuming that a surveillance system is in place), the probability of introducing SHB to a free area is below 0.2 if the number of consignments moved is lower than 100 and the prevalence in the shipped matrix at the place of origin is below 0.05 (Figure 5C). Considering an intermediate case, in which the test sensitivity is 0.5 (a testing system is in place, but detection of SHB might be affected by other factors which could be expected to reduce the probability of detection), the probability of introducing SHB into an SHB-free area is below 0.9 if the number of consignments is lower than 100 and the prevalence in the shipped matrix at the place of origin is below 0.05 (Figure 5B).



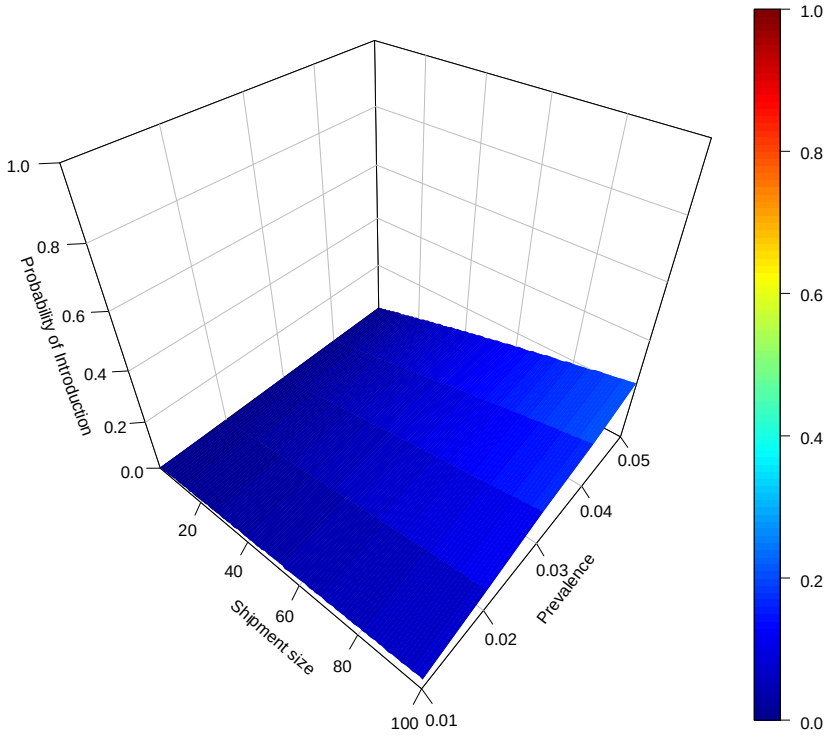
(A) Test sensitivity is equal to 0

[13] A package consists of 1–2.5 kg of adult bees, with or without a queen, and usually accompanied by a can of sugar syrup, contained in a ventilated shipping case. Even when testing is in place the probability of detecting SHB is nearly zero given the nature of the type of shipment under consideration.

[14] Which was based on the prevalence value used by the OIE and used only for illustration purposes.



(B) Test sensitivity is equal to 0.5



(C) Test sensitivity is equal to 0.95

Figure 5: Probability of SHB introduction into a SHB-free country as a function of the shipment size and SHB prevalence in the shipped matrix at the place of origin, considering a test sensitivity of 0 (A), 0.5 (B) or 0.95 (C), reflecting situations where testing is completely ineffective or absent, reduced or high, respectively

Tables 1 and 2 show the number of shipments needed to reach a probability of introducing SHB of 0.05 (Table 1) or 0.95 (Table 2), at the three different test sensitivities (0, 0.5 and 0.95) and at SHB prevalence ranging from 0.01 to 0.05. For example, the number of consignments needed to ensure that the risk of SHB introduction arising from intra-EU movement of queens from an SHB-infested area to an SHB-free area (test sensitivity 0.95) is ≤ 0.05 (5%) would range from 20 to 102 depending on the assumed prevalence of SHB (bottom row of Table 1). However, movement of around 1 140 queen consignments from an area with an SHB prevalence of 0.05 will result in a probability of introduction of SHB of 0.95 (bottom right cell of Table 2).

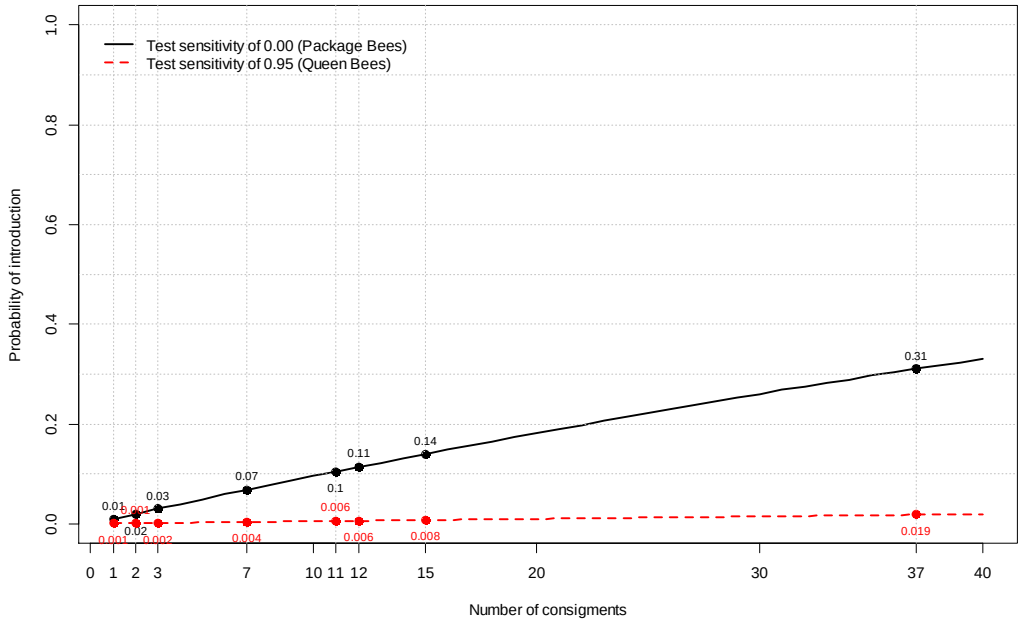
Table 1: Number of consignments that need to be moved from an SHB-infested to an SHB-free area to achieve a probability of SHB introduction of 0.05

Test sensitivity	SHB prevalence				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0.00	6	3	2	2	1
0.50	11	6	4	3	2
0.95	102	51	34	25	20

Table 2: Number of consignments that need to be moved from an SHB-infested to a SHB-free area to achieve a probability of SHB introduction of 0.95

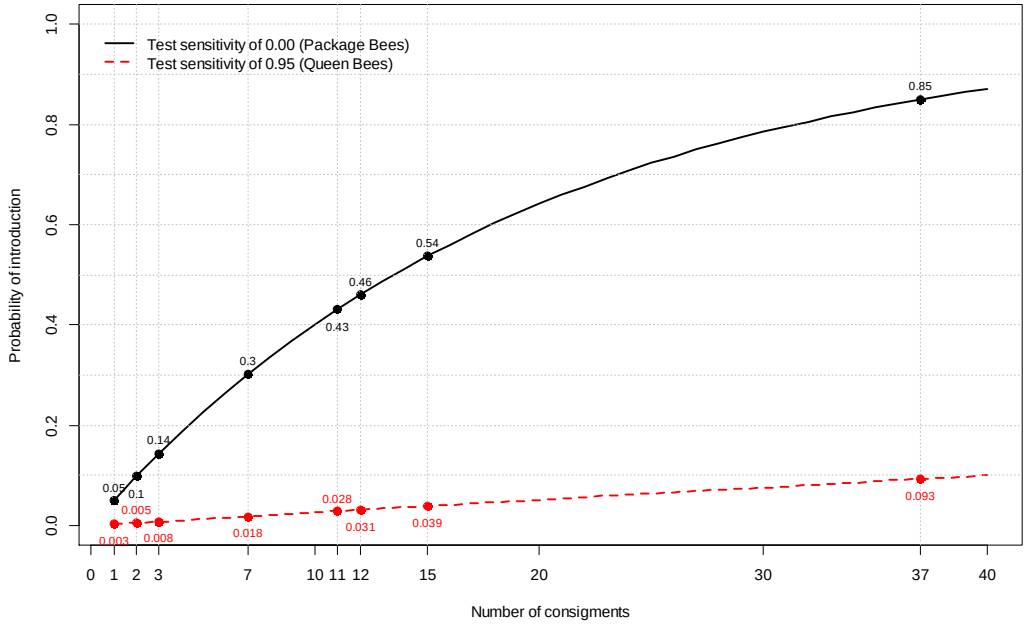
Test sensitivity	SHB prevalence				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0.00	299	149	99	74	59
0.50	595	296	196	146	116
0.95	5 934	2 938	1 939	1 440	1 140

Figures 6 and 7 present the probability of SHB introduction using the officially reported number of bee consignments exported from Italy to other EU countries in 2014 (14 MS, numbers ranging between 1 and 37, assuming that all exported consignments originated from the infested region for a given SHB prevalence) (see Appendix C). In a country receiving 37 consignments of bees, and assuming a test sensitivity of 0 (e.g. movement of package bees), the probability of introducing SHB, if the bees originate from an area with a SHB prevalence of 0.01 (Figure 6) or 0.05 (Figure 7), is 0.31 and 0.85 respectively. If, however, the test sensitivity for the 37 consignments is 0.95 (e.g. movement of queen bees), the probability of introducing SHB if the bees originate from an area with an SHB prevalence of 0.01 (Figure 6) or 0.05 (Figure 7) is 0.019 and 0.093, respectively.



Every dot on the graph represents an officially reported number of bee consignments shipped from Italy to another European country in 2014 (see Appendix C) and the number next to it represents the probability of SHB introduction assuming test sensitivity 0 (e.g. package bees; black full line) or test sensitivity 0.95 (e.g. queen bees; red dashed line).

Figure 6: Probability of SHB introduction into a SHB-free country given **SHB prevalence at the place of origin of 1%** as a function of the number of consignments and the sensitivity of the applied test



Every dot on the graph represents an officially reported number of bee consignments shipped from Italy to another European country in 2014 (see Appendix C) and the number next to it represents the probability of SHB introduction assuming test sensitivity 0 (e.g. package bees; black full line) or test sensitivity 0.95 (e.g. queen bees; red dashed line).

Figure 7: Probability of SHB introduction into a SHB-free country given **SHB prevalence at the place of origin of 5%** as a function of the number of consignments and the sensitivity of the applied test

The results described in this section indicate that the probability of SHB introduction is mainly dependent on the sensitivity of the test to detect SHB in consignments and the number of shipments arriving in a country in a given time period. However, it is important to highlight that the scenarios presented here are hypothetical, with the aim of estimating potential risks under different circumstances. More detailed data on the actual SHB prevalence in the area of origin, the sensitivities of the test used and number of consignments shipped between MS would be required to provide estimates that reflect the real situation.

3.3. Mitigation measures reducing the risk of SHB transmission via consignments

This section describes a practical assessment of 'monitoring SHB freedom of a consignment', 'isolating the consignment' and 'treating to prevent SHB infestation in a consignment' (see Sections 3.3.1, 3.3.2 and 3.3.3 and Table 4) since these risk mitigation measures were identified in the scientific report on SHB as being highly effective and feasible to reduce the risk of SHB transmission for consignments of live bees or non-living bee products[15] and materials (EFSA, 2015). Some of these measures are included in the current OIE recommendations (OIE, 2015) for importation of honey bee products and used beekeeping equipment, as summarised in Table 3. When authorising import or transit of the commodities, Veterinary Authorities should ensure that the conditions prescribed and relevant to the SHB status of the honey bee and bumble bee population of the exporting country or zone are met. Except for the recommendation that the commodities should come from apiaries situated in a country or zone free from SHB, all the other OIE recommendations imply a physical treatment (heating, freezing, freeze drying, irradiation, or filtering) to guarantee freedom from SHB.

Table 3: OIE recommendations (2015) for importation of honey bee products and used beekeeping equipment

Matrix	Recommendations
Bee-collected pollen	Comes from apiaries situated in a country or zone free from SHB OR contains no live bees or bee brood; AND has been treated to ensure the destruction of SHB, in conformity with one of the following procedures: (i) freezing at core temperature of –12°C or less for at least 24 hours; OR (ii) irradiation with 400 Gy; OR (iii) desiccation by freeze drying or equivalent; OR (iv) by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; AND all precautions have been taken to prevent contamination with SHB
Beeswax/ propolis	Come from apiaries situated in a country or zone free from SHB; OR contain no live bees or bee brood AND are processed propolis or processed beeswax; OR contain no live bees or bee brood; AND have been treated to ensure the destruction of SHB, in conformity with one of the following procedures: (i) freezing at core temperature of –12°C or less for at least 24 hours; OR ii) irradiation with 400 Gy; or (iii) by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; AND all precautions have been taken to prevent contamination with SHB.
Royal jelly	Comes from apiaries situated in a country or zone free from SHB; OR is encapsulated for human consumption; OR has been treated to ensure the destruction of SHB, in conformity with one of the following procedures: (i) heating to 50°C core temperature and holding at that temperature for 24 hours; OR (ii) freezing at core temperature of –12°C or less for at least 24 hours; OR (iii) desiccation by freeze drying or equivalent; or (iv) irradiation with 400 Gy; OR (v) by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; AND all precautions have been taken to prevent contamination with SHB

[15] The assessment focuses on bee products to be used in apiculture, including, for example, bee-collected pollen, unprocessed comb honey, fresh royal jelly, propolis with beeswax, comb beeswax and brood combs.

Matrix	Recommendations
Honey	Come from apiaries situated in a country or zone free from *A. tumida*, OR has been strained through a filter of pore size no greater than 0.42 mm; OR has been treated to ensure the destruction of *A. tumida*, in conformity with one of the following procedures: (i) heating to 50°C core temperature and holding at that temperature for 24 hours; OR (ii) freezing at core temperature of –12°C or less for at least 24 hours; OR (iii) irradiation with 400 Gy; OR (iv) by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; AND all precautions have been taken to prevent contamination with *A. tumida*
Used beekeeping equipment	Come from apiaries situated in a country or zone free from SHB OR has been thoroughly cleaned, and treated to ensure the destruction of SHB, in conformity with one of the following procedures: (i) heating to 50°C core temperature and holding at that temperature for 24 hours; OR (ii) freezing at core temperature of –12°C or less for at least 24 hours; OR (iii) irradiation with 400 Gy; OR i(v) by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing AND all precautions have been taken to prevent contamination with SHB

The sections below include information on OIE-recommended methods and, if available, the scientific evidence underpinning these. Furthermore, it is important to note that Commission Implementing Decision 2015/838/EU considers only non-extracted comb honey intended for human consumption as an at-risk honey consignment; extracted honey is not considered as a consignment at risk since SHB cannot survive the extraction procedure.

Table 4: Scoring of effectiveness (Eff.), technical feasibility (Feas.) and uncertainty (Unc.) of methods to monitor, isolate or treat a consignment. Measures are highlighted in green when they have a high score for effectiveness, a high score for technical feasibility and low score for uncertainty.

Risk mitigation measure	Place	Queens and attendants			Colonies/swarms or package bees			Bee products to be used in apiculture			Non-extracted comb honey			Used beekeeping equipment		
		Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.	Eff.	Feas.	Unc.
Monitoring SHB freedom in a consignment																
Visual inspection and health certificate*	O, D	H	H	L	M/L	M/L	H/H	M	L	H	M	L	H	M	L	H
Isolating the consignment																
Use of fine mesh	T	H	H	L	H	H	L	H	H-M-L	L	H	H-M-L	L	H	H-M-L	L
Treatment to prevent SHB infestation in a consignment																
Fumigants	O	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	U	U	U
Irradiation (400 Gy)[(a)]	O	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	H	L	L
Freezing (–12°C or less at core for at least 24 hours)[(a)]	O	NA	NA	NA	NA	NA	NA	H	H	L	H	M	L	H	H-M-L	L
Heating (50°C at core for at least 24 h)[(a)]	O	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	H	H-M-L	L
Desiccation	O	NA	NA	NA	NA	NA	NA	H	H	L	NA	NA	NA	H	H-M	L

(a): Recommended by OIE Terrestrial Animal Health Code (2015).
NA, not applicable; L, low; M, moderate; H, high; U, unknown; O, place of origin; T, during transport; D, place of destination.

3.3.1. Monitoring SHB freedom of a consignment

This section assesses how different consignments can be monitored for SHB freedom. Detection of SHB at the place of origin, during transport or at the place of destination should be notified immediately to the competent authorities.

Visual inspection and health certificate

The earlier the life stage of SHB, the more difficult it will be to detect it by visual inspection. Visual inspection of queens and attendants is considered highly effective and technically feasible, whereas this is medium for colonies and low for swarms and package bees, respectively (Table 4). If a consignment is deemed to be SHB negative, a health certificate stating that no SHB is present should be issued by an authorised person (e.g. Veterinary Services) when the queen and attendants are collected and packaged into a cage at the place of origin. Queens should be caged individually with their attendants to allow proper inspection. However, it is permissible for one box to contain many cages, each with one queen and its attendants. The use of a box with many cages (battery cage style), with each cage containing one queen but many attendants free in the box, should be discouraged because in these cases proper control both at the place origin and destination cannot be guaranteed. It is also important to highlight that OIE Code and the current regulation concerning importations from third countries (Commission Regulation (EU) No 206/2010) state that this mode of transportation is not allowed. The application of a fine mesh (2 mm size) around the consignment immediately after inspection will guarantee adequate protection and maintain SHB freedom during transport (see below Section 3.3.2.). At the place of destination, the queen is visually inspected and, if negative, transferred to a new container containing new sugar candy and new attendants, while the attendants remaining in the consignment should be kept under sanitary restriction, and properly inspected under biosafety conditions in order to prevent any spread of SHB if it is detected. The attendants from the consignment should be killed and visually inspected in accordance with the OIE Manual for diagnostic tests and vaccines for terrestrial animals (OIE, 2015). This is described in the legislation on import of queens from third countries (Directive 92/65/EEC). Bees, bee products, non-extracted comb honey and used beekeeping equipment should also be visually inspected to detect the presence of SHB both at the place of origin and at the place of destination. It is recommended that beekeepers repeat visual inspections and use SHB traps for at least 4–8 weeks following arrival of a consignment, in particular in the case of swarms and package bees.

The technical feasibility of the visual inspection method is high for queen bee consignments given that the consignment will contain only one queen and a maximum of 20 attendants[16] (Table 4). The technical feasibility and effectiveness are moderate for colonies since many more bees are present (and distributed throughout the combs of the colony) and even lower for package bees and swarms as finding SHB is difficult in consignments containing only bees at high density in the package container (Table 4). Visual inspection of bee products, non-extracted comb honey or used beekeeping equipment has in general a low technical feasibility and medium effectiveness since SHB could easily hide. The uncertainty on the scoring is high since both technical feasibility and effectiveness are dependent on the size of the commodity (Table 4). A further limitation could be the fact that the person checking the non-bee commodities at the place of destination is unlikely to be expert in visual detection of SHB.

The assessment based on the assumption of a perfect implementation of the visual inspection, although this might not always be the case in practice. Therefore, the SHB status of the area of origin of consignments should also be considered in the health certificate for intra-EU movement of bee consignments, as is already done for import from third countries. Concerning the movement of live worker and drone bees with or without associated brood combs, the Veterinary Authorities of receiving countries should require the presentation of an international veterinary certificate attesting that the bees come from apiaries situated in a country or zone free from *A. tumida* (OIE, 2015). According to Council Directive 92/65/EEC and Regulation 206/2010/EU, which regulates import, bees and bumblebees shall come from an area of at least 100 km radius which is not the subject of any restrictions associated with SHB and where this infestation is absent.

[16] These numbers are described in the legislation on import of queens (Regulation 206/2010/EU, Directive 92/65/EEC).

Registration

Registration on the location of apiaries and number of hives is crucial to maintain effective monitoring of SHB freedom. Such a register, together with tracking information on the travel route of shipments, is also essential to facilitate investigation of outbreaks. It is still impossible to obtain a complete set of data on European beekeeping because of the high variability of colony registration requirements in the MS. Even if colony registration is mandatory, registration of beekeepers and colony numbers is still not accurate in some countries. Information on the beekeeping industry should be based on the compulsory registration of each beekeeper and honey bee colony (Chauzat et al., 2013). Some data on trade in bees and bee products between MS are already submitted to TRACES (see Appendix C for an example on bees), but it is assumed that this is only a fraction of the true trade. It is recommended that beekeepers keep records of their trade activities, even in the absence of a national registration system.

3.3.2. Isolating the consignment

Use of fine mesh

Immediately after visual inspection at the place of origin, the consignment should be packed in a fine mesh (maximum 2 mm size[17]) that is properly sealed to guarantee pest freedom during transport. This measure is highly feasible and highly effective for consignments of living bees since they are generally small (Table 4). A queen can survive a cage with attendants in for up to 10 days with only limited discomfort. However, this confined environment is not very comfortable for queens and time in this situation should be kept as short as possible. It should be assumed that queens are collected, inserted into the cages with attendants and kept in a box until a certain number of queens have been caged. Then the final package is arranged and wrapped up with the fine mesh. This process should be carried out within 24 hours before departure of the shipment.

In the case of bee products, non-extracted honey and used beekeeping equipment, the effectiveness remains high but the feasibility will range from high to medium or low depending on the size of the shipment (e.g. depends on the degree of overlap between different mesh bands, width of the mesh role or availability of the material) (Table 4). Extracted honey is stored in tanks or drums of different size and should be visually inspected. Once the containers have been sealed, the application of a fine mesh should guarantee enough protection from intrusion of SHB. Beeswax is rendered and refined. These processes are sufficient to destroy SHB. Beeswax cakes are placed on pallets and should be visually inspected immediately before the application of fine mesh. Beeswax cakes can be transported in pallets or containers depending on the size of the shipment. Non-extracted comb honey can be placed in hive bodies or supers piled up on pallets. It can be protected with the application of a fine mesh as soon as the pallet has been loaded. Used beekeeping equipment can be uploaded on pallets and protected by SHB contacts/incursion by the application of a fine mesh. It can be transported on pallets or in containers according to the size of the shipment. The use of fine mesh to wrap up used beekeeping equipment is recommended also when it is placed within a container. In general, in the case of non-living bee products, other non-penetrable material can be used (e.g. plastic sheets, foils) or these may already be packaged in non-penetrable containers at the end of their processing. For instance, pollen stored at –20°C or dehydrated is already packed in sealed plastic bags (fit for food) or containers. The mesh should be stored at –12°C or lower for at least 24 hours after use.

3.3.3. Treatments to prevent SHB infestation in a consignment

Implementation of treatments is recommended at the place of origin to prevent possible introduction of SHB into new areas.

Fumigants

The use of fumigants should be considered only for consignments of used beekeeping equipment as they will destroy living consignments and negatively affect bee products and non-extracted comb honey (Table 4). However, no fumigant is currently authorised in all MS. Sulphur dioxide is easy to use, low cost and relatively safe, but ideal concentration and exposure times are not known.

[17] On average, SHB adults are larger than 2 mm (Ellis et al., 2002; Stedman, 2006).

Furthermore, sulphur dioxide is not approved for use in any MS as a Product Type 18 for the control of arthropods by means other than repulsion or attraction (Regulation 528/2012/EU). Approval can be granted by a MS to protect animal health if it can be proved that no other treatments are capable of doing the job. Once 120 days have passed since the registration request, a comprehensive dossier is required before approval can be extended. It can therefore be expected that authorisation to use sulphur dioxide to eradicate SHB in commodities is unlikely.

However, it might be useful to further investigate the applicability of fumigation as this measure is successfully used against insects in, for instance, plant commodities[18]. No literature is available on the application of gaseous ozone (O_3) to sanitise consignments for SHB, although it has been used against other insects. It is highly oxidative and unstable and decomposes rapidly to oxygen without leaving residues. It is a powerful disinfectant used for water treatment and in the food industry (USPA, 1999) and has recently attracted increasing interest for control of insect pests in stored grain (Tiwari et al., 2010). Several papers report on the potential of ozone to control stored-product pests (see Hansen et al., 2012, for a literature review). Gaseous ozone has already been demonstrated to be effective against some honey bee pests and pathogens. For example, neonates and adults of the greater wax moth (*Galleria mellonella*) (Lepidoptera: Pyralidae) were killed in few hours while eggs required 48 hours at the concentration of 460–920 mg O_3/m^3 (James, 2011).

Irradiation

Irradiation cannot be applied to bee consignments as it would kill the bees. Irradiation of bee products or non-extracted comb honey is not allowed under Directive 1999/2/EC. Therefore, irradiation can be applied only to consignments of used beekeeping equipment.

No data on the irradiation dose required to kill SHB are available. To date, only Downey et al. (2015) have reported studies on the radiobiology of SHB, but their aim was to determine the potential utility of sterile insect releases as a control strategy. For matings between unirradiated males and irradiated females, mean reproduction was reduced by > 99% at 45 and 60 Gy compared with controls, and no larvae were produced at 75 Gy. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture maintains an online database of literature on irradiation of organisms for sterile insect technique (SIT) and phytosanitary irradiation (PI) (Bakri et al., 2005; IAEA, 2012).

Irradiation has been described to kill other insects. Johnson (1987) treated raisins infested with three different ages of larvae of dried-fruit beetle (DFB) *Carpophilus hemipterus* L. (Coleoptera: Nitidulidae) with gamma radiation at doses of 130–798 Gy. Pupae and unmated adults were also treated at 338 and 486 Gy. All doses applied to larvae prevented adult development. Only the oldest treated larvae were able to develop to the wandering stage (late third instar) before dying. Complete larval mortality occurred sooner at higher doses. Mortality of irradiated pupae was 90% at both doses and adults emerging from treated pupae died within 48 hours. Irradiated adults produced no progeny and died within 1 week after treatment.

Based on studies with 34 species in nine families of Lepidoptera, Hallman et al. (2013) suggested an efficacious dose of 400 Gy, which is the same dose as recommended by OIE Code (2015) for SHB.

Based on the scientific data presented above, it is assumed that gamma irradiation at a dose of 400 Gy will kill all SHB life stages. Studies determining the minimal required dose to kill SHB would be useful. The effectiveness, which depends on the source of irradiation and dose absorption, is considered high when applied to used beekeeping equipment (Table 4). The technical feasibility depends on the availability of facilities that can provide this service. At the moment, there is only one approved facility in (northern) Italy (see list 2015/C 51/09 for other MS[19]). It is expected that there will be limitations to the expansion of such facilities to numbers that will be required for routine implementation of this method of treatment of used beekeeping equipment, in which case the technical feasibility is low. Furthermore, routine implementation would require validation of the irradiation processes by regular dosimeter readings. This is necessary to ensure that the minimum

[18] Use of phosphine in large bulks of grain or tightly packed materials (http://www.fao.org/docrep/x5042e/x5042e0a.htm; last accessed 22 July 2015).

[19] List of approved facilities for the treatment of foods and food ingredients with ionising radiation in the MS. OJ C 51, 13.2.2015. p. 59–63.

dose delivered is efficacious and at the same time that the dose is lower than the maximum dose tolerated by the consignment.

Freezing

As mentioned in the scientific report (EFSA, 2015), no precise threshold values for each developmental stage are documented. However, SHB adults, eggs and larvae are assumed to die at temperature/time combinations of –9°C for 30 minutes, –1°C for 1 hour and between 1°C and 4°C for 8 days, once all the material is at the target temperature (Stedman, 2006). The effectiveness of freezing is high when a core temperature of –12°C or less for at least 24 hours is achieved (OIE, 2015). This measure has a high technical feasibility for application to bee products such as pollen and royal jelly and moderate feasibility for non-extracted comb honey (Table 4). In the case of the latter, the feasibility strongly depends on the size of the batch. It cannot be applied to live bees because they would be killed at this temperature. The feasibility of this method for used beekeeping equipment is highly dependent on the size of the equipment. Cooling trucks able to freeze the loaded materials could be used (Dietemann and Lerch, 2015) since they can easily reach SHB-infested areas, avoiding any transportation of infested equipment to the treatment facility.

Heating

Heating to 50°C core temperature and holding at that temperature for 24 hours is recommended for non-living materials by the OIE Code (OIE, 2015), whereas Annand (2011) reported the successful killing of SHB adults in an insect-proof room in less than 18 hours at 45°C. It is important to reduce relative humidity below 50%, otherwise elevating the temperature might result in increased larval activity and damage (Stedman, 2006). Heating is highly effective and its technical feasibility when applied to used beekeeping equipment (unless wax is present) varies from high to low given that many beekeepers cannot apply this procedure in their own apiary (Table 4). Ovens able to contain beekeeping equipment are not currently available, and it seems unlikely that they will be routinely available in the future. This measure is not applicable to live bees, non-extracted honey combs or bee products. Honey will melt. Pollen is usually stored frozen or air-dried; and treatment at 50°C for 24 hours is not compatible with the preservation of its nutritional quality. Beeswax has a relatively low melting point range of 62–64°C. Heat treatment to 50°C is achieved by the routine rendering process of beeswax.

Desiccation (freeze-drying or equivalent)

As mentioned in the scientific report (EFSA, 2015), SHB development can also be prevented by maintaining a low relative humidity (RH) of 34% or below (presentation Pettis, as reported by Somerville, 2003; Annand, 2011) since SHB eggs do not hatch, dry and die. This can be achieved using dehumidifiers in closed rooms, the use of fans to provide air movement through the equipment or by storing equipment allowing a good air flow through it (Pettis, as quoted by Somerville, 2003; Annand, 2008). It is highly feasible to use of dehumidifier in the honey house or places where used beekeeping material is stored (Table 4). According to OIE (2015), desiccation by freeze-drying or equivalent is sufficient to destroy SHB in pollen and royal jelly. There are no data available, but it is assumed that water elimination is incompatible with the survival of SHB. Freezing cannot be applied to living bees. The effectiveness is considered high if adequate RH levels are maintained. The time required to achieve 34% RH will depend on the local environmental conditions. Further research on different SHB life stages could help to provide guidance on the time required. Furthermore, the concept of 'desiccation', meaning elimination of all water (dehydration), is not suitable for non-extracted comb honey or used beekeeping equipment.

3.4. Mitigation measures managing SHB infestations in apiaries in infested areas where eradication is no longer the objective

Two years later after SHB introduction in the USA, the beetle was well established in the country and caused considerable damage to apiculture in Florida (estimated cost around $ 3 million only in 1998; Ellis et al., 2002). Questionnaires to beekeepers in Queensland (Australia) indicated losses ranging from 6.87% to 12% of the bee hives in the summers 2008–2009 till 2013–2014 (see Table 5). This is a very conservative estimate based only on the actual losses reported by the 50% of registered

beekeepers who responded in these surveys (Diana Leemon, Department of Agriculture and Fisheries, University of Queensland, Australia, personal communication, 17 November 2015). This section addresses which mitigation measures could be implemented in case SHB would be considered endemic within the EU.

Table 5: Summary of hive losses reported in Queensland (Australia) SHB surveys

Hive category	2008–09	2009–10	2010–11	2013–14
1-5	22%	8%	23%	9%
6-20	14%	14%	22%	11%
21-49	11%	12%	16%	6%
50-499	8%	10%	15%	9%
≥500	4%	7%	6%	4%
Total responses	1,455	1,230	1,341	480
Total hives	67,309	56,257	54,303	14,210
Overall losses reported	4,777/67,309	5,316/56,259	6,348/54,303	976/14,210
Overall % loss	**7.10**	**9.44**	**12**	**6.87**

The mitigation measures considered are monitoring the pest status in an apiary, good beekeeping practices, honey house management, mechanical control, veterinary medicines and biocides, and soil treatment. These measures are described in detail in the Sections 3.4.1 to 3.4.6. In practice, implementation of mitigation is often done by combining different measures, as described in Section 3.4.7. Finally, additional risk mitigation factors that may be applied in controlled environments for queen producing, as well as for movement control, are described (see Sections 3.4.8 and 3.4.9).

3.4.1. Monitoring the pest status in an apiary

Different methods can be used to monitor the pest status at the apiary level in order to manage SHB infestation in an infested area where eradication is no longer the objective. Diagnosis of SHB infestation requires some experience and a good knowledge of the beetle's life cycle.

Several methods are available to detect adults, larvae and eggs of the SHB in living honey bees, such as visual inspection and use of traps. PCR detection on hive debris could also be used for SHB detection in hives. Soil investigation is the only method which can be used to detect pupae in the soil. The sensitivity of some of these methods has been already evaluated in the EFSA scientific report on SHB (EFSA, 2015). In order to monitor SHB infestation throughout the year, a combination of these methods should be applied.

SHB populations seem to peak in autumn in the USA and Australia and decline in spring (Frake et al., 2009; de Guzman et al., 2010; Annand, 2011). So far, all the SHB-infested apiaries found in Italy have been found in late summer/autumn both in 2014 and in 2015, suggesting some seasonality of SHB detection. More experience is required to determine whether or not current field conditions (ongoing surveillance, restriction and eradication measures) influence the epidemiological data. It has been shown that SHB abundance is significantly correlated with the proportion of hot days,[20] leading to a peak of infestation in autumn in south-eastern USA (de Guzman et al., 2010). A study of the relation between SHB population build-up and the environmental conditions (particularly temperature) in Italy would improve our understanding.

Identification of each of the SHB life stages can be achieved by morphological examination. Criteria for diagnosis are described in the OIE Terrestrial Manual (OIE, 2015) and in the COLOSS Beebook paper concerning SHB (Neumann et al., 2013). Morphological identification is sufficient for adult SHB. In the differential diagnosis of SHB, other nitidulid beetles should be considered, such as *Cychramus luteus* (Neumann and Ritter, 2004), *Carpophilus lugubris* (Marini et al., 2013) and several other species of the same family (Mutinelli et al., 2015a). Images of other Nitidulidae beetles species found on rotten fruits in the Calabria region can be consulted on the IZSVe website.[21] Diagnosis by PCR is

[20] Maximum environmental temperature ≥ 27 °C.
[21] http://www.izsvenezie.com/documents/reference-laboratories/beekeeping/aethina-tumida/documentation/aethina-tumida-in-rotten-fruits-in-calabria.pdf (last accessed 22 July 2015).

required when SHB larvae, pupae, eggs and/or damaged adults are found, particularly in the case of first detection if adult SHB are not present (Chauzat et al., 2015).

The text below provides practical information concerning the different methods available to conduct quick diagnosis of the infestation. Traps can also be used to monitor SHB infestation and are described in the Section 3.4.4, 'Mechanical control'.

Visual inspection

This method is suitable for detecting adults, larvae, eggs and damage from SHB in hives, in stored frames and equipment, or in apicultural facilities. Visual inspection is easy to conduct but is time-consuming, needs concentration and requires experience and good knowledge of SHB behaviour and localisation inside the hive and in apicultural facilities. The sensitivity of visual inspection is dependent on the rigour of inspection (EFSA, 2015).

SHB adults are located everywhere in the hive, and preferentially in places where they can hide from honey bees. A large part of the SHB adult population seems to prefer bottom boards (Lundie, 1940; Neumann et al., 2013). Adult beetles move very quickly and have an aversion to daylight; therefore it is crucial to be very rapid during the inspection. In cold conditions, SHB adults are more likely present within the bee cluster to take advantage of heat produced by honey bees. Outside hives, they can be found in honey houses and beekeeping equipment.

Larvae are observed in aggregations on and in the combs containing brood, pollen or honey and on the bottom board in the debris (Neumann et al., 2013).

Wandering larvae are attracted to light and can be found exiting the hive to pupate, typically during early evening (Stedman, 2006). Most often the pupation occurs in the first 2 m around the colony (Pettis and Shimanuki, 2000), but larvae can migrate considerable distances to find suitable soil (Stedman, 2006). They can also be found in stored honey combs.

Eggs are difficult to detect by visual inspection. Generally laid in clusters of 10–30, they are quite small (approximately 1.4 x 0.26 mm (length x with), two-thirds of honey bee eggs; OIE, 2015). They are laid inside cell combs (brood cells or sealed honey combs) or in small cracks and crevices and around the supporting edges of frames. They will either be present or not, depending on the defensive behaviour of honey bees. The more hygienic the bees are, the less is the chance eggs will be present, as they are detected and removed (Neumann and Härtel, 2004; Spiewok and Neumann, 2006; Ellis and Delaplane, 2008).

The OIE Terrestrial Manual (2015) and the Coloss Beebook (chapter concerning SHB research; Neumann et al., 2013) describe a method to be used to detect SHB adults and larvae. This procedure relies on shaking all honey bees present in the hive onto a sheet of opaque plastic, preferably light in colour, or plywood. The beetle is detected by attentive visual examination. All frames should also be bounced against the plywood to dislodge adult beetles from the comb. After all frames have been examined, the empty box should be bounced on the sheet to remove the remaining SHB. The same procedure should be applied to supers and the bottom board of the colony. The bees accumulated on the sheet can be bounced off it in front of the reassembled hive. This time-consuming method of investigation is hard to conduct for routine diagnosis of SHB at the apiary level because it can induce disorder and robbing (OIE, 2015).

The methods currently used for routine diagnosis in infested areas, for instance in Italy, are easier to carry out (Stedman, 2006; Mutinelli et al., 2014; Zawislak, 2014). Anyone can carry out hive inspection with the aim of detecting SHB if trained to manipulate a beehive. This method of carrying out inspections is the most feasible in the field. It requires a certain minimum training and awareness of SHB biology and morphology to correctly check the hives and quickly detect and recognise damage caused by the different life stages of the beetle. Colony inspection begins right at the entrance of the hive. It relies on the rapid but meticulous examination of the lid, the inner cover, the frames and the bottom board:

1) Remove the lid and check for the presence of adult SHB running away.
2) Remove the inner cover and check both sides. Check also the top of the frames for running SHB adults.

3) Remove the frames from the hive one by one. Each side of the frame should be quickly observed to check the presence of adult SHB, larvae, eggs and damage. The first frame can be left outside the body of the hive to make it easier to handle the other frames. Subsequent frames should be put back into the body or super to prevent robbing in the apiary during the examination.

4) Beetles can hide inside the cells of combs. It is also important to examine the lid, the bottom board, the side faces, corners, interstices of the hive and hive components.

If robbing is unlikely, the super can be examined by placing it on the inverted lid of the hive in a sunny spot. Adults will escape from the sunlight and retreat down into the lid. After about 10 minutes, the presence of adult SHB in the lid can be checked by lifting the super (Zawislak, 2014).

If there is a risk of robbing, the super should be inspected in the same way as the body of the hive, i.e. comb by comb, by replacing each frame in the box after its examination. During the examination of the body, the super can be placed on a reversed lid, so that no bees or SHB can escape (Spiewok et al., 2007).

In order to improve the sensitivity of the visual inspection, the hive can first be removed from its original position, then opened and replaced by an empty hive (Spiewok et al., 2007; Neumann and Hoffmann, 2008). Each frame is then removed and examined for SHB for the first time. The honey bees are then shaken into an empty box and the comb inspected for a second time for SHB, this time in absence of bees, before being placed into the new hive. Once all the frames have been examined, the original hive box and bottom board are inspected. However, this method is time-consuming, and requires additional beekeeping equipment and therefore is not suitable for routine monitoring of SHB infestation in large apiaries. It could be recommended for health certification, in order to demonstrate the absence of SHB infestation in a colony, or for research purposes.

Another method for screening hives, originally described in Canada, uses a white 12-litre bucket fitted with a wire-mesh screen (about 6 mm) fitted halfway down the depth of the bucket. The bottom of the bucket is covered with a thin layer of vegetable oil. The frames are shaken inside the bucket and bees are stopped unharmed by the wire-mesh whereas beetles fall into the vegetable oil. Field data suggest that this method is more sensitive than simple visual inspection when the infestation level is low.[22] However, it is time-consuming and there is a high risk of inducing disorder and robbing in the apiary.

In cases of severe and advanced infestation, damage may be observed during inspection of the colony or the apicultural facilities: combs of honey with a glistening and slimy appearance, or fermentation of stored honey by yeast introduced by adult beetle defecation, causing odour described as that of decaying oranges. In the most severe cases, honey can run out of the cells and out of the hive entrance (Figure 8).

Beekeeping equipment and stored honeycombs before extraction must be also inspected because they are attractive to SHB. These materials can be inspected visually. UV lights can also be used to attract, detect and control larvae and adult beetles in the honey house (Duehl et al., 2012).

In general, visual inspection is a good method for SHB diagnosis and makes it possible to evaluate the severity of the infestation (for example, level of infestation, presence of several life stages of SHB or of damage). However, it is time-consuming, and requires the hives to be opened, and therefore cannot be conducted all year round (depending on weather conditions). Moreover, detection of SHB can be difficult in cases of low infestation. Baiting and trapping are useful and rapid tools to improve detection performance when used together with visual inspection (see Section 3.4.4 on 'Mechanical control'). In the 2014 Italian outbreak (September–December), almost no beetles were caught in traps, which could be due to the very low infestation level and/or the autumn season (Mutinelli et al., 2015b). Later in the Italian outbreak, in September 2015, SHB adults were found in Beetle Blaster™ traps, thus confirming that traps should be used in addition to visual inspection. However, traps can never replace visual inspection.

[22] http://www.omafra.gov.on.ca/english/food/inspection/bees/2011-shb-report.htm

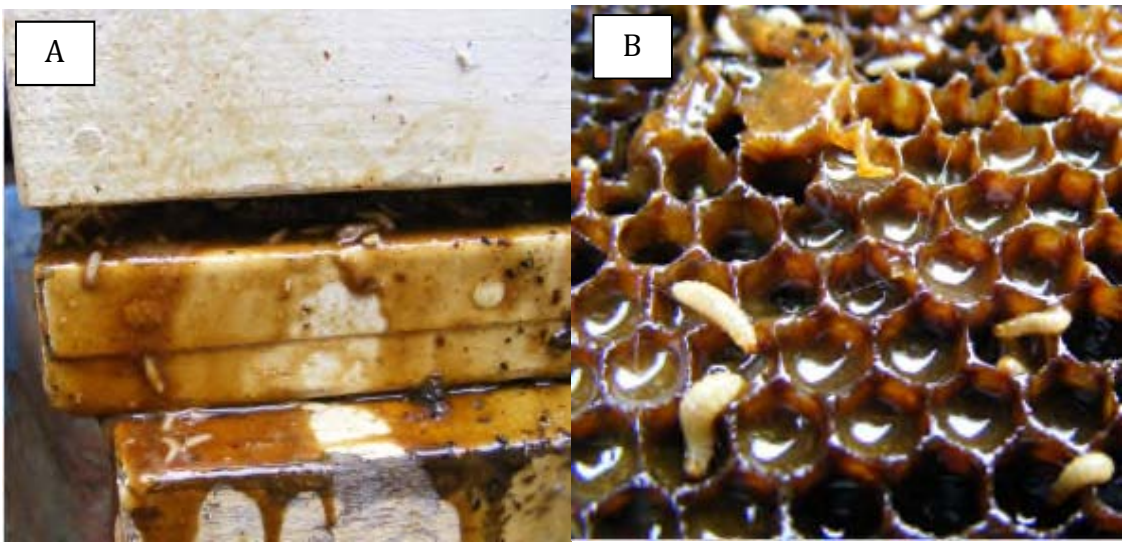


Figure 8: (A) Slime running out of a hive destroyed by SHB and (B) SHB larvae on slimed up honey frame

PCR on debris

Another method for SHB detection in a hive is PCR analysis of debris collected on the bottom boards (Ward et al., 2007; Cepero et al., 2014). Hives need to be equipped with a bottom board floor to collect the debris.

This method could be used to monitor SHB in 'free' areas. In particular, it has been used in hive debris collected from apiaries in a Spanish surveillance study (Cepero et al., 2014) and is used in surveillance in the United Kingdom (Mike Brown, National Bee Unit, United Kingdom, personal communication, 12 November 2015). However, in the context of SHB infestations when eradication is no longer the objective, it may not be very appropriate as a tool to monitor SHB infestation for management purposes, as it cannot distinguish dead and live SHB stages. PCR is very sensitive and detects SHB DNA. In case many samples need to be analysed, bulking them up for molecular analysis is just one of the approaches to use. However, a positive result does not necessarily mean that the apiary is currently infested. Even if sanitation measures have been applied, DNA could remain in the material and PCR could detect it. It is clear that visual inspection of hives would be required to check if the apiary is still infested.

PCR on hive debris needs still to be validated in field conditions in order to better evaluate its performance. A standardisation of the sampling procedure should be also conducted (EFSA, 2015). In the event of positive results, apiary inspections and further investigations must be conducted to check for the presence of SHB or for traces of its passage (e.g. visual examination of colonies, traps, visual examinations of debris).

Soil examination

Pupation occurs in the soil in the vicinity of the colonies. The majority of wandering larvae and pupae are found within 0.90–1.80 m of the hive (Pettis and Shimanuki, 2000) if suitable soil is present, but distance may increase drastically (to 200 m or more) if there is no suitable soil around the hive (Stedman, 2006). Beetle larvae, pupae and newly emerged adults are mostly found in the first 20 cm of the soil (Pettis and Shimanuki, 2000; de Guzman et al., 2009).

Digging and sieving soil around infested hives is the only method available to screen for SHB pupae. These investigations could give information concerning the state of development of the infestation in the apiary. However, soil examination is time-consuming and not always easy to do, notably depending on the size of the apiary and the type of the soil.

3.4.2. Good beekeeping practices

Ensuring strong, healthy, well-populated colonies with a young productive queen is very important within a SHB-infested area where eradication is no longer the objective. Weak or queen-less colonies should be destroyed or merged with strong, healthy, well-populated colonies that are less susceptible to SHB infestation (Mustafa et al., 2014). Unoccupied space in the hives for the bees to manage should be limited, so that bees occupy both sides of the comb with little room left unoccupied. This can limit the development of the SHB population in the hive (Ellis, 2005; OMAFRA, 2010[23]; Hood, 2011).

Under conditions of high temperature and high humidity, even strong hives can be quickly taken over by SHB larvae if there are enough SHB in the hive. Under these conditions, the number of times the hive is disturbed should be limited, as SHB can react to disturbances in the hive by quickly laying large numbers of eggs with the result that the bees are overwhelmed by the large numbers of larvae and cannot manage them. It appears that under these conditions bees may abscond from the hive, leaving it to the SHB larvae (Ellis et al., 2003; Neumann and Elzen, 2004).

Colonies should be managed to maximise their strength, minimise the area of undefended frames per colony and minimise hive disorganisation. Large numbers of feral honey bee colonies have allowed SHB to become established in the wild in areas with suitable climatic conditions in Australia (Spiewok et al., 2008). Infested colonies must be managed carefully to prevent swarming as a result of colony splitting, as this will limit the presence of swarms (feral colony) in the environment. When splits are made, the number of bees left behind should be sufficient to protect the brood and to prevent SHB egg laying and larval damage. Colony splitting has been carried out in the infested protection zone (20 km radius) in Calabria. Around 3 000 swarms were produced to prevent natural swarming[24] between April and September 2015. All these swarms were examined[25] for SHB and found to be negative. Approximately 300 natural swarms were recovered during April and May 2015 and found to be negative for SHB.

Apiaries should be regularly checked. Bees are able to clean up some infested frames (Annand, 2011), but when a colony is highly infested with SHB larvae, as indicated by the presence of damaged combs, it should be destroyed by killing the bees and subsequent burning of the hive. In this case, the honey may have started to ferment as a result of the actions of the yeast *Kodamaea ohmeri*, which is associated with SHB (Leemon, 2012). If a SHB infestation results in the fermentation of hive products (called 'slime'), caution must be taken when cleaning hive equipment as slime could be a potential health risk to immunocompromised or immunosuppressed people. This yeast has also been recovered from a broad variety of sources (e.g. flowers, mushrooms, in association with insects) (Lachance and Kurtzman, 2011). A small number of infections and deaths in severely immunocompromised subjects, both young and old, have recently been attributed to *K. ohmeri* fungaemia. The latest reports are from Italy (Santino et al., 2012), but they are not related with the recent detection of SHB. In addition to treating hive equipment covered in slime with a 1% bleach solution (sodium hypochlorite), appropriate protective equipment such as waterproof gloves and a face shield should be worn when handling any slimed bee equipment. These precautions are essential for operators with suppressed immunity to minimise the potential exposure to the yeast *K. ohmeri*.

Bleach (sodium hypochlorite) has been used to disinfect beekeeping material that has been in contact with highly infested material (slimy material). It kills the yeast that causes the fermentation of the honey but does not kill SHB. It is a fast-acting and safe product suitable for controlling SHB larvae in honey houses and for use in the salvage of combs infested with larvae. Bleach-treated combs remain repellent for at least 24 hours (Park et al., 2002). After treating with a 1% bleach solution (sodium hypochlorite), infested frames can be washed with water if they are to be re-used.

3.4.3. Honey house management

Good beekeeping practices should be applied in the management of honey houses in order eliminate SHB and/or prevent the development of SHB infestation and subsequent ruin of stored honey frames through the action of SHB larvae (OMAFRA, 2010; Hood, 2011).

[23] http://www.omafra.gov.on.ca/english/food/inspection/bees/11rep.htm#small (last accessed 5 November 2015).
[24] 0010658-23/04/2015-DGSAF-COD_UO-P: swarm production in the protection zone—Calabria region.
[25] Swarms were put into a hive and inspected visually a few days later.

Honey should be extracted immediately from supers (no longer than 2 to 3 days) once removed from colonies. Only remove as many honey frames as can be extracted without the need for longer storage.

If possible, manage honey bee colonies using queen excluders[26] and, if not using queen excluders, avoid bringing any honey bee brood into the honey house in honey supers. SHB larvae preferentially breed on brood and pollen.

Good honey house hygiene should be maintained and houses should be kept as clean as possible. Any residues of honey processing should be removed, particularly capping wax, honey and slumgum, since eggs and larvae present in supers could colonise the habitat and undergo hatching and further development. Adult SHB are attracted to the odour of the wax and honey when these are being extracted, and they will fly towards the honey house and try to gain entry, particularly around dusk. Adult SHB will lay eggs in any suitable materials lying around the honey house. Be vigilant for adult SHB flying into the honey house around dusk.

The extraction facility should be cleaned thoroughly as soon as the majority of extraction is complete.

Honey supers, extracted frames and unused honey supers should be stored in a freezer or in a cool room (ideally below 10°C) to prevent egg hatching and larval damage. However, it is important to be aware that, if a large number of honey supers are stacked for storage, it may take a long time to chill the centre of the stack to 10°C. Remember too that adult SHB can survive low temperatures for a time by clustering together even if they cannot breed at this temperature. Annand (2011) reported that SHB adults can survive but not breed at 15 ºC so this temperature or lower would need to be maintained in the honey shed.

Alternatively, honey supers can be stored for at least 48 to 72 hours in a sealed room containing a dehumidifier to maintain a low RH of 34%; this prevents SHB development as eggs cannot survive this RH (Stedman, 2006). Fans can be used to provide air movement through the equipment, which should be stacked to allow air flow (see details in the Section 3.3.3 on desiccation). At levels of RH below 50% more than 80% of eggs fail to hatch (for a review, Cuthbertson et al., 2013).

Extracted combs should be returned to colonies to allow the bees to remove remaining honey.

In the USA, Park et al. (2002) proposed bleach as a fast-acting and safe product suitable for controlling SHB larvae in honey houses and for use in salvage of combs infested with larvae. In Australia only, fumigation of extracted supers or dead-out hives before storage is permitted (Phostoxin® or Fumitoxin®, active substance aluminium phosphide) (Levot and Haque, 2006). This is not allowed in the EU.

3.4.4. Mechanical control

As the larval development is the most damaging stage in SHB infestation, reproduction of SHB should be limited, and therefore traps should be used to reduce the number of adult SHB in an infested hive in infested areas where eradication is no longer the objective. Bernier et al. (2014) showed that implementation of in-hive traps in Canada was effective in reducing SHB populations, without compromising the bee population colony weight gain. Observations in the USA imply that intensive trapping during the rainy season could reduce the population of beetles infesting hives in subsequent seasons (Torto et al., 2010). During a field trial in Australia, an in-hive trap device was shown to be highly effective in reducing adult SHB numbers (Levot and Somerville, 2012). Traps can also be used to monitor SHB in and around the hive but should be combined with visual inspection, as mentioned in Section 3.4.1.

Different types of traps are available to capture SHB outside and inside colonies, and beekeepers are continually inventing and improving home-made traps. Trapping adult SHB outside the hive would be a smart solution, but until now no bait has been found to be more attractive than, or even as attractive as, colonies of honey bees. However, a mixture of honey and pollen, together with live adult bees (Elzen et al., 1999) or yeast-inoculated pollen dough (Torto et al., 2007a), has been shown to

[26] A selective barrier, either a sheet of perforated metal or plastic or a wire grid in a frame, is applied to the inside of the beehive with the aim of limiting the queen's access to the honey supers.

attract some[27] SHB and, therefore, baited traps may help reduce the number of invading SHB, especially during periods of higher SHB dispersal. Inside-hive traps consist of a reservoir for a killing agent, which can be entered by SHB but not by bees. The killing agent might be chemical, but could also be a liquid in which entering SHB may drown. Many inside-traps are unbaited and rely on the fact that SHB seek shelter from bee aggression, but baited traps are also used. The frequency of checking the traps depends on the season and the type of traps. Traps with smaller reservoirs need to be checked more frequently, especially during the SHB reproductive season; also, some baits lose their attractiveness over time and must be replaced (Torto et al., 2007a).

A variety of different traps and baits have been used to capture adult SHB **outside the hive**:

- Plastic bucket traps with 8-mesh hardware cloth (large enough for adult beetles to enter) glued across 7-cm-diameter holes have been used to trap SHB outside colonies (Elzen et al., 1999; Buchholz et al., 2008).
- Other traps were made of 25.5 cm PVC pipe sections with a removable cap at each end. Two openings covered with 4-mesh screen allowed beetles to enter the trap. An 18-mesh screen inverted cone, located just below the openings, funnelled SHB into the bottom cap through a small hole in the cone apex (Arbogast et al., 2007). Insecticidal strips (Vaportape II), placed in the bottom of the traps, kill trapped SHB (Arbogast et al., 2009).

Selection of the **bait** is critical to capture SHB outside the hive environment. A mixture of honey, pollen and adult bees resulted in the highest catch numbers in the field (10 g honey + 5 g pollen + a volume of 50 ml adult bees; Elzen et al., 1999), while fruits were not efficient (Buchholz et al., 2008). Arbogast et al. (2007) used pollen dough inoculated with the yeast *K. ohmeri* (Torto et al., 2007a; Benda et al., 2008) as bait and captured more SHB inside baited traps than in unbaited traps, which captured no SHB at all. Further tests with *K. ohmeri*-inoculated pollen dough showed a positive influence of shade and a negative correlation with distance to beehives, on the frequency of SHB capture (Arbogast et al., 2009). White traps (compared with black traps) **positioned** at 46 cm height (same height as colony entrances during the test) showed the highest catch numbers in the field (de Guzman et al., 2011).

Season is an important factor to consider when positioning traps, as almost half of an SHB population was observed outside the hive during the hottest month of the year while in cold seasonal conditions SHB retreated back into the hive, at least in some areas in the USA (Annand, 2011). Although traps to capture adult SHB outside the hive generally do not catch high numbers of beetles, they allow for continuous observations that provide a relative measure of SHB dispersal (de Guzman et al., 2011).

The main principle of all traps that are designed to trap SHB adults **inside colonies** is to provide passage for beetles but prevent bees from entering the traps. Oil or veterinary medicines may be used as a killing agent and/or some kind of bait to attract SHB. Cider vinegar is known to be attractive to SHB (Hood and Miller, 2003), as is pollen dough inoculated with SHB-associated yeast (Torto et al., 2007a). The usage of bait may significantly increase the number of beetles trapped (Torto et al., 2007b).

There are traps available for **all positions in the hive**: under the bottom board, on the bottom board, in the frame, as a replacement for a frame, between frame top bars and at the entrance of the hive (Neumann et al., 2013). The position of the trap is important and has to be adjusted to the different hive types and during the seasons, as the beetles tend to leave the bottom board to stay within the warmth of the clustering bees when temperatures are low. To be able to catch beetles independent of the season, it is recommended using different traps at different positions (e.g. bottom board, brood nest) simultaneously.

One trap without any bait or killing agent is made of strips of 4 mm corrugated plastic. It is easily placed on the bottom boards of the hive by sliding the strips through the flight entrance (see Appendix G). The trap is left on the bottom board for at least 2 days to give SHB some time to find the shelter. It can then be removed and thoroughly inspected without having to open the colony: each tunnel inside the strip is examined or the corrugated plastic is shaken against the sides of a bucket, possibly containing water, to immobilise adult SHB or is inserted in a plastic bag, sealed,

[27] As the number of beetles in the environment is unknown, it is not possible to give figures for efficacy. The more beetles are around, the more one is likely to catch.

shaken and directly examined for SHB. A strip- thickness of 4 mm was chosen in order to create narrow tunnels, which serve as a hiding place for SHB, but which prevent access to bees. Bottom boards should be clean as it is important to place the strip in close contact with the hive floor in order to prevent SHB hiding in any space that might remain between the trap and the floor (Schäfer et al., 2008).

The Beetle Blaster™ (http://www.betterbeetleblaster.com/) is used in Italy and is placed between frame top bars. This type of trap requires a drowning liquid (oil, vinegar, soapy water) to be added to prevent escape of the SHB from the trap once inside. It is important that the container is not completely filled (fill only to about one-third of the trap's height), otherwise beetles could escape the traps. Adult beetles enter the traps to hide from bees and are drowned in the liquid. In addition to liquids, also diatomaceous earth could be used (see Appendix G; Cribb et al., 2013). The Beetle Eater™ is a similar trap (http://www.ajsbeetleeater.com.au/).

Diatomaceous earth was assessed in field colonies using bottom board traps (Buchholz et al., 2009). The type of diatomaceous earth to use is important (only the most hydrophobic formulation tested showed any effect) and it should not be used on windy days as in this case bees can also be affected. Furthermore, the dusty diatomaceous earth was ventilated by thermoregulating bees which may thereby endanger the colony and may also reduce the quality of their products (Buchholz et al., 2009).

The West Trap™ (Hood, 2011; Zawislak, 2014) is placed on the bottom board (see Appendix G); hives must be kept level for these traps to be effective, as it contains oil in a shallow basin. The basin is covered by a screen that excludes bees. Adult beetles enter the trap from above to escape from bees and will fall into the oil and drown. West Traps™ are not suitable for use with screen bottom boards. However, colonies with screen bottom boards could work by adding an oil-filled tray below the screen. A similar trap in function is the Freeman Beetle Trap™.[28]

The Hood Trap™ attaches to a standard beehive frame[29] (see Appendix G). It has three compartments that can be filled with apple cider vinegar (as an attractant) or with oil, in which the beetles will drown as they enter. As the trap is much smaller than a frame, the entrance of the trap may not be visited as frequently as the bottom board or the top bars. There is also empty space around the trap, which bees will often fill with drone comb (Hood, 2011; Zawislak, 2014). A similar trap in function is the Beetle Jail Trap™.

The USDA beetle trap design utilises a bait of fermented pollen and a one-way exit in the bottom board, similar to a triangular bee escape, through which the beetles may pass and become trapped in an oil-filled chamber on the other side. These traps cannot be used with screen bottom boards for *Varroa* control or ventilation (Zawislak, 2014).

The Beetle Barn™ is a flat rectangular plastic trap with small openings on each side for the beetles that are too small for bees. A piece of Checkmite+™ strip (i.e. the organophosphate coumaphos, 10% w/w) is placed in a middle section and SHB that tend to hide from bees die upon contact with the strip. The trap is placed on the bottom board or on the top frame bars (Bernier et al., 2015).

Levot (2008) developed a refuge trap (Apithor™) comprising a two-piece rigid plastic shell encasing a fipronil-treated corrugated cardboard insert (Levot and Somerville, 2012). The Apithor™ trap is currently authorised only in Australia (Levot, 2008; Levot and Somerville, 2012). It is not authorised in any EU Member State and no Maximum Residue Limit (MRL) is established. Mean fiprole (fipronil plus its toxic metabolites) residues in honey ripened while the devices were in place did not exceed the limit of quantification (1 mg/kg). Two of the three wax samples contained no detectable fiprole residues. The third sample contained one metabolite at the limit of quantification (i.e. 1 µg/kg) but no other residues. This level is at least an order of magnitude lower than most allowable maximum residue limits for fipronil in foods in Australia (Levot and Somerville, 2012).

A very cheap trap being used in Australia consists of Chux® Superwipes® (www.chux.com.au), a disposable cleaning cloth (see Appendix G). The cloth is folded into approximately one-quarter to one-third the area of a bee box and placed on top of the frames in the brood box and held in place by a

[28] Examples include http://freemanbeetletrap.com/, https://www.dadant.com/catalog/product_info.php?products_id=1247 and http://www.clemson.edu/psapublishing/pages/entom/eb160.pdf (last accessed 14 September 2015).
[29] http://tigerprints.clemson.edu/all_theses/494/ (last accessed 14 September 2015).

queen excluder. The bees attack the cloth and shred parts of it, making it fibrous. When SHB are chased by bees they seek refuge in the folds of the cloth and become trapped.

Usually SHB look for shelter inside the colony to escape possible aggression by honey bees. The more the honey bees attack SHB, the more beetles will be trapped. Therefore, the efficacy of all traps is affected by the activity of the honey bees and, thus, by the strength of the colony, the level of SHB infestation, the availability of shelter inside colonies and, especially, the environmental temperatures (the activity of bees and beetles is lower at temperatures below 10–20°C).

Proper use of traps by beekeepers is important: traps should be checked regularly (every time the colony is inspected by the beekeeper in accordance with good beekeeping practice); the ability to remove propolis from traps is necessary to maintain trap function and to enable easy replacement of the trapping substance without spilling it over the bees; the trapping substance (seed oil/vinegar/soapy water/diatomaceous earth) has to be changed if the reservoir becomes overcrowded or if the substance has evaporated. If oil is used, traps should be manipulated carefully to avoid spilling oil inside the hive.

There are some examples of traps intended for the capture of larvae applied just at the colony entrance outside the hive. They should capture larvae when leaving the hive. The trap consists of two parts constructed of 3/8 inch (0.95 cm) acrylic plastic held together by catches. The lower part of the trap is watertight and half-filled with a solution of detergent and water. The upper part intercepts the larvae and is covered, except for a 3 mm gap at the level of the bottom board. Larvae enter through this gap and fall through a screen (18-gauge stainless steel wire with 2 mm openings). The screen prevents bees from falling into the detergent solution. The trap is attached to the bottom board by two 18-cm extensions on the upper part (Arbogast et al., 2012). The trap is recommended primarily as a research tool for colony-wide SHB population dynamics (Neumann et al., 2013).

Some bottom board traps (e.g. *Varroa* screen, Freeman Trap™, West Trap™) may also catch larvae that may drown in the liquid.

Wandering larvae are attracted to light, and light traps inside the honey house are successfully used by US beekeepers (Somerville, 2003). The response of SHB to different wavelengths of the light spectrum was evaluated, and 390 nm wavelength seemed most attractive to wandering larvae and adult SHB. While light traps in enclosed spaces effectively captured SHB adults and larvae, they did not capture more than control traps in the field. Therefore, light shows promise for SHB control in locations where comb is stored or honey is extracted (Duehl et al., 2012).

3.4.5. Veterinary medicines and biocides

No veterinary medicine is authorised for the control (and eradication) of SHB in the EU. However, the same veterinary medicine that is used in the USA and Canada to control SHB (Checkmite+™) is authorised in six MS (Bulgaria, Cyprus, Greece, Romania, Spain, Sweden) and Switzerland (Mutinelli, 2015) for the control of *Varroa* mite infestation. Accordingly, it would be possible to use this veterinary medicine in the other MS under 'the cascade' system.[30] The recommended duration of treatment is 14 days. This veterinary medicine has a withdrawal time of 42 days and veterinary prescription is required.

The veterinary medicine Checkmite+™ is formulated as plastic strip that is applied on the bottom board of the hive (cut in two halves) covered by a piece of corrugated cardboard or plastic (approx. 15 × 15 cm with one surface stripped off to expose corrugation) in order to create a sort of refuge trap where SHB adults can hid from bees. This refuge trap and other commercially available ones (e.g. Beetle Barn™, Bernier et al., 2015) should prevent any contact between honey bees and the coumaphos strips. Excess Checkmite+™ should be avoided since this could increase the risk of residues in beeswax and possibly honey (MRL = 100 ng/g). Checkmite+™ can be considered as a tool for the control of SHB but is not the solution to the infestation, as efficacy is not 100% and SHB may develop resistance. In fact, its efficacy rate is affected by the level of SHB infestation (more effective at higher levels of infestation), the strength of the colony (stronger colonies interact more with SHB, increasing the tendency of the beetle to look more for refuge in traps) and environmental temperature

[30] Article 11 of Directive 2001/82/EC of the European Parliament and of the Council as amended by Directive 2004/28/EC of the European Parliament and of the Council.

(at temperatures below 20°C bees are less active, with the result that SHB are less disturbed and do not need to hide). Checkmite+™ works best when the temperature within the hive exceeds 29°C; at night temperature should not drop below 21°C (Wenning, 2001).

Checkmite+™ has been reported to be kill up to 90% of the SHB adult population in honey bee colonies when the strips were placed under cardboard stapled to the hive bottom board (Elzen et al., 1999). However, in the case of whole colonies, the mean percentage mortality of adult SHB was considerably lower (53%; Neumann and Hoffmann, 2008). Baxter et al. (1999) reported efficacy of over 94% in package bees, but more than half of the 20 SHB adults experimentally introduced in the package escaped through the 10-mesh wire screening of the package and were uncounted for. Two other acaricides that are used to control *Varroa* mites also affect SHB development: the pyrethroid tau-fluvalinate (Apistan™) has been reported to be toxic to feeding and wandering larvae but innocuous to adults while botanical extracts (thymol, camphor, menthol, eucalyptol; Apilife VAR™) were toxic only to perpetually wandering larvae (Ellis and Delaplane, 2007). The current formulation of tau-fluvalinate (Apistan™) is a strip which cannot be used for SHB larvae control in the EU.

Like veterinary medicines, biocides could be used as a complementary measure to control SHB The susceptibility of SHB to selected biocides and insect growth regulators was assessed in a glass vial bioassay (Kanga and Somorin, 2012). The lethal concentration causing 50% mortality (LC_{50}) in adult SHB was 0.53, 0.53 and 0.54 µg/vial for fenitrothion, chlorpyrifos and methomyl, respectively. However, against the larval stage, fenitrothion was the most toxic, with an LC_{50} of 0.89 µg/vial. Chlorpyrifos had an LC_{50} of 1.64 µg/vial, which was similar to the LC_{50} of 1.21 µg/vial for fluvalinate and 2.24 µg/vial for methomyl. Overall, these insecticides were found to be more toxic to SHB than the organophosphate coumaphos. Among the insect growth regulators tested, fenoxycarb and methoprene were effective on early instar larvae with an LC_{50} of 30.20 and 61.89 µg/vial, respectively. However, studies of the toxicity of these biocides to honey bees are yet to be conducted (Kanga and Somorin, 2012). Biocides (**pyrethroids** in particular) are effective against any life stage of the beetle (larvae, pupae, adults) and are recommended for soil treatment (see Section 3.4.6 below).

3.4.6. Soil treatment

In an SHB-infested area where eradication is no longer the objective, soil treatment should be applied only when colony damage by SHB larvae is observed. Soil treatment with pyrethroids is intended to kill all stages of SHB possibly present (see Section 3.4.5). Based on experience in the USA, an area of 0.90–1.80 m radius around the hives should be treated to a depth of 20 cm (Pettis and Shimanuki, 2000). In Italy, a 1% solution of cypermethrin and tetramethrin was abundantly sprayed at high pressure (50 l/min) in order to drench the soil after soil ploughing (Mutinelli et al., 2014) to ensure a higher probability of exposure of SHB. In the USA, Gard Star™ (40% EC permethrin) used at label rate of 0.05% (active ingredient) is used as soil drench, especially to treat the soil underneath colonies showing severe larval development, to prevent new emerging SHB entering nearby colonies. Soil drenching with permethrin (2 ml of 0.05% solution per square inch surface area) has been shown to be highly effective against SHB larvae.[31] Following administration of the solution, the soil should appear wet (Smith et al., 2008). As these pyrethroids are toxic to bees, beehives must not be sprayed (Hood, 2011) and exposure of non-target species (e.g. feral bees) should be avoided. Therefore, it is recommended that soil treatment takes place after sunset, and other environmental considerations should be taken into account.

The use of pyrethroids for soil treatment should be considered in the framework of the EU legislation on biocidal products.[32] Biocidal products can pose risks to humans, animals and the environment owing to their intrinsic properties and associated use patterns. Therefore, biocidal products should neither be made available on the market nor used unless authorised in accordance with Regulation (EU) No 528/2012. For a biocidal product to be authorised, it must be demonstrated that the product is efficacious against the target organisms and safe for humans, animals and the environment. The assessment of active substances to be used in biocidal products according to this Regulation is

[31] Tarver et al., 2013; ESA, 87th Southeastern Branch Meeting, Baton Rouge, LA, USA (http://www.ars.usda.gov/SP2UserFiles/Place/64133000/Posters/Laboratory%20comparison%20of%20soil%20treatments%20for%20control%20of%20SHB.pdf; last accessed 30 September 2015).

[32] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012

ongoing. Among pyrethroids, deltamethrin and permethrin have already been approved for insecticide use. Where no biocidal product containing pyrethroids is authorised in a given MS to control SHB via soil treatment, the provisions in Article 55 of Regulation (EU) No 528/2012 or, where relevant, Article 56 of the Regulation, could apply.

Alternative treatments, such as treating the ground with powdered limestone, have been found to be ineffective, while slaked lime produced inconsistent results (Buchholz et al., 2009). As rain would probably reduce the efficacy of such treatment of slaked lime, more field experiments are needed to test its impact on all soil-dwelling life stages of SHB. Entomopathogenic fungi could be an alternative (Ellis et al., 2004b; Richards et al., 2005; Muerrle et al., 2006; Leemon and McMahon, 2009; Leemon, 2012). Entomopathogenic nematodes were also tested and showed high efficacy, resulting in 76–100% mortality (Cabanillas and Elzen, 2006; Ellis et al., 2010; Cuthbertson et al., 2012). However, more tests on entomopathogenic fungi and nematodes in the field are needed. Entomopathogenic fungi application to the soil is also under the scope of biocides regulation. Another alternative treatment that avoids the use of chemicals in heavily infested apiaries and which should be considered is to sterilise soil using high-pressure steam. This system has been used in strawberry fields in California to control soil weeds, pathogens and pests. Temperatures above 50 ºC can be maintained for 30 minutes (Fennimore et al., 2014) at 25 cm depth. Steam could be also combined with solarisation to increase effectivity (Samtani et al., 2012). However, the steaming approach, like the use of insecticides, is not selective and could therefore also have a strong environmental impact. Anaerobic soil disinfestation, which consists in using the by-products of anaerobic metabolism, obtained by covering organic matter with air-tight plastic, has been recently considered as an alternative to soil chemical sterilisation to control potato cyst nematode (Streminska et al., 2014). However, none of those systems have been tested against another Coleoptera pest or SHB. Another option is to remove the upper level (at least 20 cm) of the affected soil and subject it to heat or freezing treatment or desiccation in appropriate facilities (if available) to kill all SHB pupae. These last examples are potentially new control avenues to be considered. They have not yet been analysed and no data are available so far, but these approaches would avoid environmental contamination and technology used in other fields (e.g. fruits) could probably be used.

3.4.7. Overview of routine SHB monitoring and management in an apiary in a SHB infested area where eradication is no longer the objective

Sections 3.4.1–3.4.6 describe individual control measures whereas this section describes their practical implementation by combining different control measures within in an apiary in an SHB-infested area where eradication is no longer the objective (Figure 9). The pest status has to be monitored routinely by visual inspection of hives, apiary facilities and equipment. Traps should be used as an additional method to detect SHB and implementation of good beekeeping practices is crucial. As soon as SHB is detected, routine management should be reinforced by strict implementation of good beekeeping practices, monitoring must be intensified and the need for any action should to control SHB infestation in the beehive should be considered. The implementation of control measures has to be considered taking into account the general health status of the infested colony, since adult SHB populations are not necessarily harmful to healthy honey bee colonies (OIE, 2015). The entire colony has to be checked for SHB damage (mainly caused by SHB larvae). If comb damage is detected, destruction of the colony must be considered and a decision has to be made if the equipment should be sanitised or destroyed. The soil around the hive could be treated if it is assumed to be infested and if a biocide is authorised within the concerned MS.

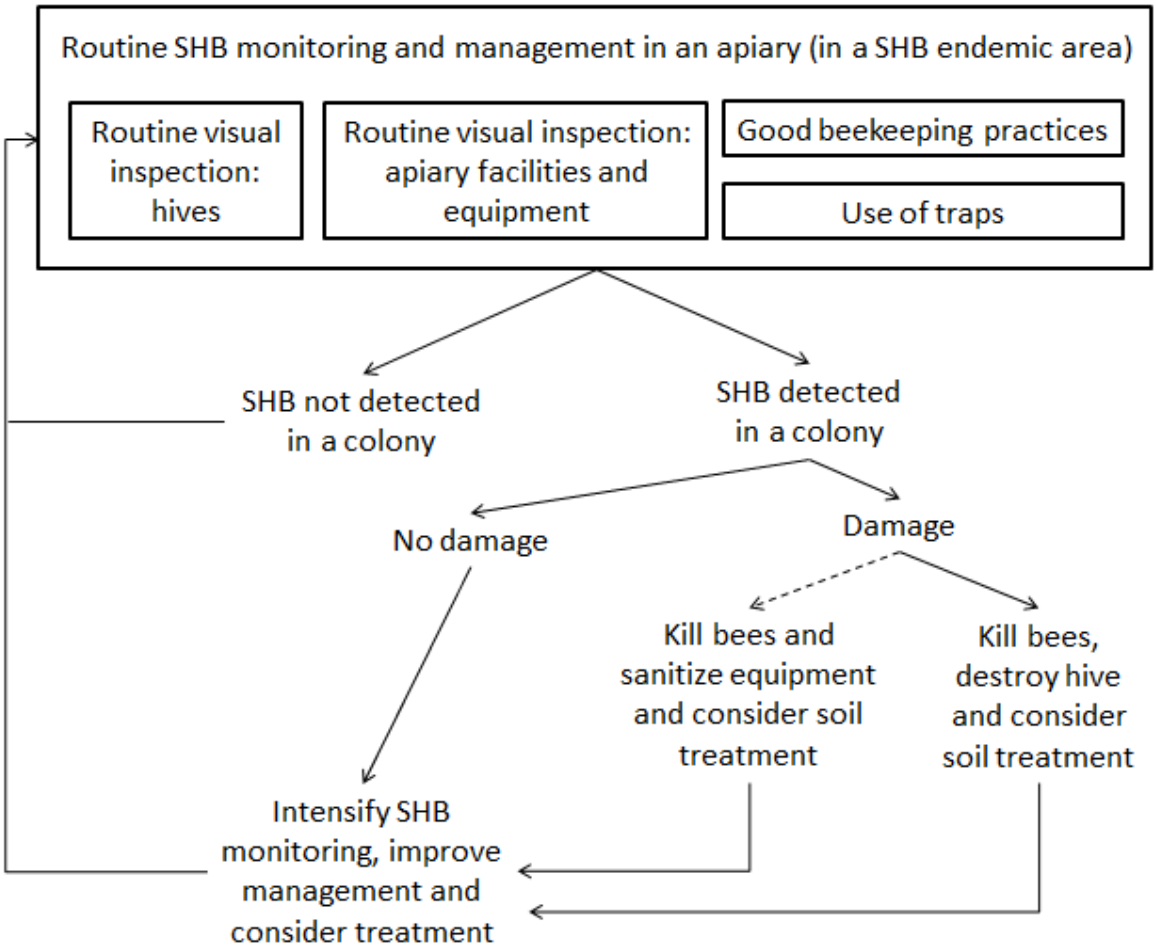


Figure 9: Overview of routine SHB monitoring and management in an apiary in an SHB-infested area where eradication is no longer the objective

3.4.8. Additional risk mitigating factors that may be applied in controlled environments for queen producing

As mentioned in the scientific report (EFSA, 2015), it is impossible to implement a closed system as used to rear bumblebee colonies due differences in the biology of these two species. Therefore, there are no specific measures applicable that could guarantee SHB freedom in a controlled environment for honey bee queen rearing. The following mitigating factors could be considered only to reduce the probability of SHB infestations and facilitate early detection in honey bee queen-rearing environments:

- registration of the queen-rearing apiary and of all apiaries belonging to the same beekeeper and of those within a radius of at least 15 km
- regular inspection of the apiary and facilities by the competent veterinary authority
- inspection of all colonies used for queen rearing
- recording of all goods entering the facilities (e.g. live bees, feed, equipment).

3.4.9. Movement control

Whereas the measures described above can be applied at the apiary level, movement control has to be applied in a larger geographical area. As mentioned in the EFSA scientific report (2015), the Italian competent authorities implemented a protection zone[33] and the surveillance zone[34] defined as (in

[33] Movements of bees and commodities are allowed only from 30 days after the last confirmed positive result (detection of SHB).

absence of an EU standard) the territory within a radius of 20 km and 100 km,[35] respectively, around an SHB-confirmed apiary. No movement of honey bees and bumblebees or commodities (unprocessed apiculture by-products, beekeeping equipment and comb honey intended for human consumption) is allowed from the whole territory of Calabria and Sicily to other zones in the EU (Commission Implementing Decision 2014/909/EU of 12 December 2014).

Similar to outbreak situations, there is no EU legislation in place regarding movement control of honey bees, bumblebees or commodities within an SHB-infested area. It is recommended that restrictions on the movement of bees and commodities from infested to non-infested areas be maintained until SHB is eradicated, to prevent spread of the pest to pest-free areas within the territory of the same or another MS.

3.5. SHB surveillance

3.5.1. SHB surveillance in a SHB-infested area

Effect of radius on the probability of SHB to escape the surveillance zone

The probability of an outbreak escaping a surveillance zone covering a radius ranging from 0 to 200 km was explored using the analytical approach described in Schley et al. (2009). The results are presented in Figure 10 using estimates for the spread of SHB obtained using the distance-only model. The median probability that an outbreak would spread beyond a surveillance zone with a radius of 100 km (i.e. the size of surveillance zone implemented around the index case by the Italian authorities following the detection of SHB[36]) is 0.027 (0.95 credible interval (CI) 0.019–0.041). Reducing the radius of the surveillance zone to 50 km increases this probability to 0.053 (0.95 CI 0.037–0.08). A similar trend was obtained from simulations using both the 'distance-only' and 'distance and ownership' spread models (see Section 3.1 and Appendix B). The probabilities of escape for simulations of the 'distance-only' model were estimated to be 0.126 (0.95 CI 0.055–0.20) and 0.0003 (95 CI 0–0.005) at 50 km and 100 km, respectively. However, there is a discrepancy between the analytical and simulation results for radii below 40 km. This is a consequence of an absence of apiaries to the north, south and east of the infested area (Figure 10), limiting spread at these distances in the simulation model, which was not taken into account in the analytical approach. It is important to note that the results described in this section incorporate the effects of control measures in place, including movement restrictions and destruction of infested apiaries (see Appendix B for details).

When choosing the radius for the surveillance zone, it is necessary to balance two competing factors. A smaller radius might allow more intensive surveillance and, hence, increase the likelihood of detecting infested apiaries within the surveillance zone. It could also make controls on movements within the zone more feasible. However, a smaller radius also increases the likelihood of a SHB escaping the surveillance zone into a region that is not under surveillance, possibly delaying detection.

[34] Movements of bees and commodities are allowed only after inspection of the apiary whereas movements of bees and commodities is allowed after two consecutive health inspections carried out 21 days apart with negative results demonstrating absence of SHB.
[35] Based on import legislation (Directive 92/65/EEC and Regulation (EU) no 206/2010).
[36] According to the order issued by the region of Calabria (number 94, 19 September 2014).

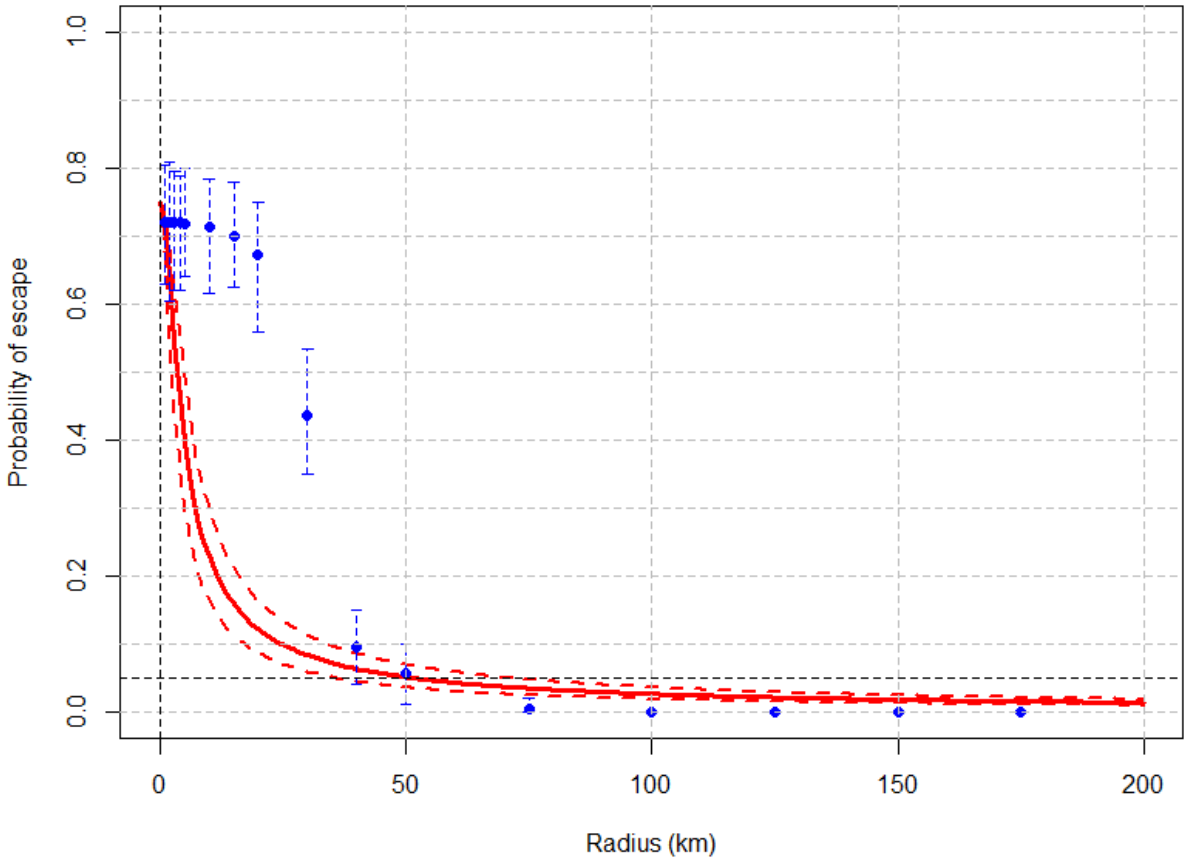


Figure 10: Probability of SHB escaping a surveillance zone of a given radius using the analytical approach described by Schley et al. (2009). The solid line represents the median and the dashed lines represent the 95% credible interval. The blue circles and error bars show the median and 95% credible interval from simulations of the 'distance-only' model

Criteria for regaining SHB-free status

The OIE Animal Health Terrestrial Code (2010) provides the requirements for 'officially' free status as a result of an eradication programme (Article 9.4.4.2(c)). In particular, for 5 years following the last report of SHB, an annual survey must be carried out on a representative sample of apiaries in the country or zone to provide 95% confidence of detecting SHB if at least 1% of apiaries were infested at a within-apiary prevalence rate of at least 5% of hives. Such surveys may be targeted towards areas at higher risk of infestation.

There are relatively few data available to determine the appropriate number of years before 'official' freedom from SHB can reliably be declared. The 5-year requirement is based on the biological characteristics of SHB. Survival of adult beetles depends on environmental conditions such as temperature and humidity, but, in practice, adult female beetles can live for at least 6 months and potentially for over 1 year (de Guzman et al., 2012). Although adult beetles are attracted to bee colonies to reproduce, they are able to survive and reproduce independently in other environments and using other food sources (for instance certain types of fruit, as shown in experimental conditions). A substantial portion (75%) of the SHB life cycle is spent in the pupal stage in soil, though there are no published data on how long pupae are able to survive in soil. In addition, it is not known if, or for how long and at what level, SHB could persist in potential reservoir populations of feral bees and bumblebees. Consequently, any recommendation for the number of years required before 'official' freedom from SHB can be declared is subject to high uncertainty. However, the longer the period for which SHB is not detected, the greater the confidence that an area has regained freedom.

In order to design the sampling frame of the surveillance required to regain 'officially' free status, it is essential to have information on (i) test sensitivity; (ii) the probability of introduction of SHB to the

country or zone; and (iii) the number and location of apiaries and the number of hives per apiary in the country or zone. When designing the survey it is reasonable to assume that identification of SHB infestation is 100% specific, given the uniqueness of SHB natural history and morphology and the availability of a confirmatory PCR test (Ward et al., 2007), if required.

When surveillance is carried out over a 5-year period, it is appropriate to analyse the results of each annual survey in a stepwise manner (Figure 11). In this context, the results of surveillance from previous years is used to calculate the prior probability of freedom from SHB (Prior P_{free}), which is updated based on surveillance for the current year (to produce Posterior P_{free}). Finally, this is adjusted to allow for the possibility of reintroduction of SHB to the area (to produce Posterior P_{free} adjusted). This approach is a natural way to integrate the surveillance data for each year, rather than treating the annual surveys as independent sets of observations.

The risk-based estimate of system sensitivity tool (RiBESS) developed by EFSA (2012) provides a framework for designing surveys to demonstrate freedom from infection, including stepwise updating of the probability of freedom, and can be applied to SHB.

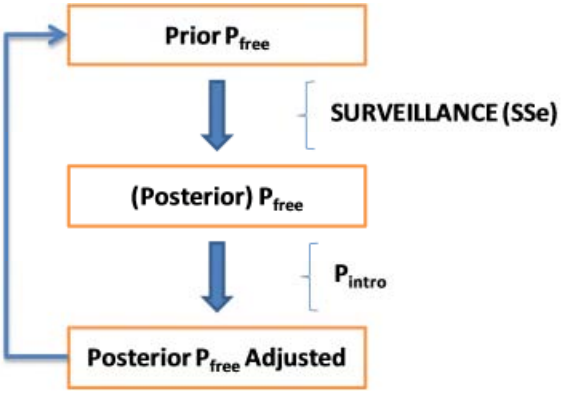


Figure 11: Stepwise analysis of historical surveillance data for SHB. SSe is the sensitivity of the surveillance system and P_{intro} is the probability of introduction to the region

3.5.2. SHB surveillance in a SHB-free area

In addition to the Italian regions Calabria and Sicily,[37] other territories of the EU historically have SHB-free status. Notification of the pest is implemented in the whole EU[38] and initiatives have been taken to increase awareness and training to encourage reporting of all cases suggestive of SHB infestation. Continued efforts to increase the availability of beekeepers and veterinary inspectors trained to recognise SHB and laboratories able to analyse suspected samples would further facilitate early detection of the pest. Recommendations on SHB surveillance are described in the guidelines which have been elaborated by the European Union Reference Laboratory for Honeybee Health (EURL) (Chauzat et al., 2015). As different modalities for surveillance could be implemented, each MS should consider which type of surveillance is best suited for its current situation.

3.6. Role of kept bumble bees to host and spread SHB

Several characteristics have to be fulfilled for a species to act as a host for SHB. The availability of suitable food resources and the temperature and humidity conditions are fundamental parameters for SHB development and survival. Moreover, the attractiveness of the host and its defence mechanisms against SHB determine the quality of the host.

Some experiments have been conducted in order to evaluate the susceptibility of bumblebees to SHB. They have quite exclusively been performed on *Bombus impatiens*, the main species reared for the crop pollination in North America (Velthuis and Van Doorn, 2006). One study includes also some investigations on *B. pensylvanicus* feral colonies in the USA (Graham et al., 2011a). In Europe, the

[37] Commission implementing decision 2015/838/EU.
[38] Annex I to Council Directive 92/65/EEC.

native species *B. terrestris* is kept for pollination. No experiments are reported on *B. terrestris* as a host for SHB. Nevertheless, extrapolation of the results on *B. impatiens* to *B. terrestris* could probably be done as these two species present some common characteristics. They belong to two subgenera, *Bombus* s.s. and *Pyrobombus* for *B. terrestris* and *B. impatiens* respectively, which are so-called pollen storers (Velthuis et al., 2006). In particular, they produce large and long-lived colonies, with stored honey, pollen and wax (Goulson, 2010). *B. terrestris* is the main species used for pollination worldwide (Europe, North Africa, Asia, Australasia and South America). It has a wide natural distribution (all over Europe, in coastal North Africa and in West and Central Asia), produces colonies of about 200–400 workers and adapts quite well to artificial conditions. Several subspecies of *B. terrestris* are used for pollination; they differ in their coloration. *B. terrestris dalmatinus,* having superior rearing characteristics, is the dominant subspecies in the pollination industry and often used outside its natural distribution area. *B. impatiens* is used only in North America. It originates from the eastern part of the subcontinent. *B. impatiens* colonies include about 300 to 500 workers at its peak and therefore are a little bit bigger than *B. terrestris* colonies (Velthuis et al., 2006).

3.6.1. Food resources within bumblebee colonies

As studies on SHB rearing have revealed, the foodstuffs on which the adults and larvae feed do not have to be very specific, as long as they contain sufficient proteins (i.e. pollen, bee brood) for egg production and larval growth, and a source of carbohydrates. Honey appears to be important to beetle longevity although honey alone is not sufficient for offspring production. SHB could also reproduce on fruits alone, at least under laboratory conditions (Ellis et al., 2002).

Like honey bees, *B. impatiens* and *B. terrestris* colonies store nectar and pollen (Goulson, 2010). Because of the size of the bumblebee colonies and of the biological peculiarities of these species, food collection and storage are less quantitatively relevant than in honey bees because they do not overwinter (Goulson, 2010). Nevertheless, protein foraging by SHB is possible in bumblebee nests (Ambrose et al., 2000), and SHB has been found to naturally infest and reproduce in commercial bumblebee colonies in the field (*B. impatiens*) in North America (Spiewok and Neumann, 2006), demonstrating that food resources in bumblebee colonies are suitable for SHB development.

Experimental studies have shown that bumblebee colonies (*B. impatiens)* are suitable for SHB reproduction (Hoffmann et al., 2008). Indeed, adult beetles appear to be able to invade the colony, locate the food store and feed on protein diet (i.e. bee bread), to mate and to oviposit.

3.6.2. Temperature and humidity conditions in bumble bee colonies for SHB development and survival

Development of SHB is known to be mainly affected by temperature and humidity. Temperature affects oviposition, hatch success, time to hatching and larval growth. Minimum temperature for development was estimated at 13.5°C for eggs, 10°C for larvae and pupae (Meikle and Patt, 2011). Annand (2011) found that temperature below 15°C and above 45°C prevents oviposition and that relative humidity below 34% prevents eggs survival. Temperature also influences adult longevity, which appears to be maximum at 28–32°C (Meikle and Patt, 2011).

Bumblebee species exhibit nest homeostasis, tightly regulating the temperature within the nest at around 30°C (Goulson, 2010), a temperature that appears to be highly suitable for SHB development and survival. It is important nevertheless to consider that a particularity of most bumblebee species is that they have an annual life cycle. The survival of the colony is based only on queens, generated in autumn, which mate, hibernate and emerge in late winter or spring to found a new nest and lay eggs to produce workers. In conclusion, temperature and humidity conditions within a bumblebee colony are suitable for the survival of SHB, except during winter.

3.6.3. Attractiveness of bumblebee colonies to SHB

Bumblebees have similar biological traits to honey bees: nectar and pollen collection and storage, and wax comb construction (Goulson, 2010). Ambrose et al. (2000) demonstrated that experimental infestation of bumblebee colonies with SHB was possible. Under laboratory conditions, SHB is indeed able to complete an entire life cycle in bumblebee colonies. Natural infestation of commercial bumblebees (*B. impatiens*) has been observed also in the field in colonies installed near infested

honey bee apiaries for research purposes (Spiewok et al., 2006). Releasing honey bee colonies (*A. m. ligustica*) and bumblebee colonies (*B. impatiens*) in a greenhouse to study transmission and host choice of SHB revealed that the level of infestation of bumblebee colonies was not significantly different from honey bee colonies (Hoffmann et al., 2008). However, no field data on SHB infestation in natural bumblebee colonies are reported.

Another experiment was conducted with the aim of determining if adult SHB which have emerged from the soil are attracted by neighbouring host colonies or conduct long-range dispersal flights. *B. impatiens* colonies and *A. mellifera* colonies were alternatively placed in a circle of about 15 m radius. SHB adults released in the centre of the circle seem to prefer *B. impatiens* over *A. mellifera.* However, the results need to be interpreted with caution because most of the SHB did not enter in any host colony but left the apiary, supporting that the theory that SHB initially conduct long-range dispersal flights (Neumann et al., 2012).

Some studies have focused on what is or could be attractive to SHB in bumblebee colonies. Spiewok and Neumann (2006) found that SHB were attracted to bumblebee workers and bumblebee-collected pollen. Graham et al. (2011b) demonstrated that adult bumblebees, stored pollen, brood and wax volatiles are attractive to SHB. In another experiment, *Kodamaea ohmeri,* a yeast known to attract SHB by producing volatiles, was detected in commercial *B. impatiens* colonies and in feral *B. pensylvanicus* colonies free from SHB (Graham et al., 2011a), suggesting that the presence of this yeast may facilitate bumblebee colony infestation.

Taken together, there is scientific evidence that SHB is attracted to bumblebee colonies, at least under controlled conditions. However, there are insufficient data available to assess whether there is a difference in the attraction of SHB to bumble bees compared with honey bees.

3.6.4. Host defence mechanisms against SHB

Different social defence behaviours against the SHB have been observed in honey bee colonies: social encapsulation, removal of beetle eggs and larvae, aggression, absconding (Neumann and Elzen, 2004). In experimental conditions, bumblebee colonies (*B. impatiens*) , like honey bees, exhibit defensive behaviours against SHB (Ambrose et al., 2000; Stanghellini et al., 2000; Hoffmann et al., 2008): removal of SHB life stages (eggs and larvae), sting, investigating and attacking behaviours. Nevertheless, these defensive behaviours do not appear to be enough to prevent the infestation and the reproduction of SHB in bumblebee colonies.

3.6.5. Capability of kept bumblebees to spread SHB

Commercial bumblebee colonies used for pollination are produced under stringent confined conditions that prevent SHB infestation. The bumblebee colonies inside the production facilities are completely isolated from the outside world. The production takes place in laboratory-style controlled environment. Furthermore, colonies are investigated for common parasites and infectious agents of bumblebees (e.g. *Nosema bombi, Crythidia bombi, Locustacarus* (*Bombacarus*) *buchneri* and *Apicystis bombi)*. Materials and surfaces are regularly disinfected to prevent any contamination between colonies. Escaped bumblebees are collected and destroyed; they are never taken back to the colonies.[39] All inputs and outputs are monitored and controlled (e.g. pollen disinfected using cobalt irradiation). There is no direct contact with honey bees or wild bumblebees. The whole production process is guided by standard operational procedures and very strict quality and laboratory protocols.

According to EU legislation,[40] bumblebee shipments must come from authorised facilities which are under strict surveillance of the competent authorities. They must come from an area free from SHB. Consignments coming from third countries shall consist of containers of bumblebees, each containing a colony of a maximum of 200 adults. They must be visually inspected for absence of disease and infestation, and accompanied by a health certificate; bumblebee delivery is traceable in the EU Traces system (see details in Appendix F). Consequently, the risk of introducing SHB from bumblebee colonies coming from production units can be considered low.

[39] http://www-pub.iaea.org/mtcd/meetings/PDFplus/2010/38586/Presentations/AMRQC12_0065.pdf (last accessed 15 September 2015).

[40] Commission Regulation (EU) No 2010/206 for third country importations; Council Directive 92/65/EEC, amended by Commission Decision No 2010/270/UE for intra-EU trade.

Bumblebee colonies could become infested during transport to their destination if shipment conditions are not protective enough to prevent adult beetles entering the packages. Bumblebee colonies could be indeed attractive for SHB (see Section 3.6.3). The risk of SHB survival on *Bombus* spp. during transport can be considered as high, since adult SHB are able to survive between 5 and 9 days without food and water (Pettis and Shimanuki, 2000). The use of fine mesh could prevent entry of SHB into consignments during transport (see Section 3.3.2).

Once they reach their destination, bumblebee colonies are transferred to greenhouses and tunnels for crop pollination. Greenhouses and tunnels are not strictly confined. In some cases, bumblebee colonies can also be used in open-field conditions. Infestation during pollination services is possible because bumblebees can act as a potential host for SHB (see Sections 3.6.1 to 3.6.4). After their release for pollination service, bumblebee colonies are not inspected and, therefore, in the event of an SHB infestation, it would not be detected. In addition, after pollination services, colonies and boxes are not always properly destroyed and disposed of and may be abandoned; they could act as a host for SHB multiplication and a source of contamination. Any food sources remaining in the box (wax, pollen) could support SHB survival. Therefore, it is recommended to destroy the bumblebee boxes after the pollination service. In Italy, this has been made official with an order of the Ministry of Health.[41]

Commercial bumblebee species do not swarm (Goulson, 2010), so they cannot disseminate adult SHB in natural conditions. Moreover, because of their annual life cycle, they cannot host adult SHB during the cold season.

4. Conclusions

TOR 1: The risk of survival, spread and establishment of SHB in and from Calabria and Sicily into other parts of Italy and the EU

- There is a lack of detailed epidemiological data on the Italian outbreak that would allow insights into introduction, survival, spread and establishment, in particular regarding a systematic analysis of all apiaries around infested ones over time, tracking of data on movements of bees, bee products or used beekeeping equipment, description of environmental conditions and presence of potential reservoirs such as feral bees and bumblebees.
- Movement of an infested hive could spread SHB rapidly over large distances. Modelling SHB spread in the absence of movement of hives suggests that, with natural spread alone, the beetle alone will take more than 100 years to reach Abruzzo from Calabria (around 250 km northwards). A model considering the ownership of multiple apiaries per beekeeper indicated a 10-times-faster spread.
- From the modelling, using data provided from September 2014 to September 2015, with a credible interval of 95%, it can be inferred that the infestation has not been eradicated in Calabria. This has been confirmed by further detections in October and November 2015.
- Opportunity maps based on calculated soil temperature at 20 cm depth indicate that, once introduced, the SHB could complete its life cycle in all Member States between May and September.
- The probability of SHB introduction is mainly dependent on the sensitivity of the test to detect SHB in traded consignments and the number of shipments arriving in a country in a given time period. Implementing a sensitive SHB testing scheme in consignments could reduce the probability of SHB introduction around 20-fold compared with absence of SHB testing. Given that the SHB prevalence in an area is dependent on the control measures in place, the probability of introduction will increase when SHB prevalence increases, which could be from 2.5 to 7 times larger if no SHB testing is in place. The lack of reliable information on these parameters did not allow concrete estimations reflecting the field situation.

[41] 0015320-09/06/2015-DGSAF-COD_UO-P.

TOR 2: risk mitigating factors that could potentially be effective in ensuring safe intra-EU trade of live bees, apiculture products and by-products as regards the transmission of SHB

- Detection of SHB by visual inspection and subsequent delivery of a health certificate, within 24 hours before dispatch, is highly effective and feasible for consignments of queen bees only.
- Bee colony data are incomplete or inconsistently recorded across Europe. Use of fine mesh with maximum 2 mm pore size to avoid contamination during transport is highly effective and feasible for consignments of bees, bee products, non-extracted comb honey and used beekeeping equipment. However, feasibility decreases as a function of the size of the consignment. Currently, implementation of precautions to prevent contamination of the shipment is required only for import from third countries.
- For consignments of bee products to be used in apiculture, implementation of freezing is highly effective and highly feasible to reduce the risk of SHB transmission.
- For consignments of used beekeeping equipment, implementation of freezing, heating or desiccation is highly effective to reduce the risk of SHB transmission. The feasibility greatly depends on the size of the shipment and on the facilities available.

TOR3: risk-mitigating factors and methods in apiaries, alternative to currently employed complete destruction of the apiary and additional risk-mitigating factors that may be applied in controlled environments for queen producing

- Monitoring infestation in apiaries will help SHB control. Visual inspection is the preferred method to detect SHB in apiaries where eradication is no longer the objective. Different life stages of the pest and the presence of damage can be detected depending upon the expertise and rigour of inspection.
- Traps and PCR analysis of hive debris are other methods that can be used in apiaries where eradication is no longer the objective and in addition to visual inspection. Furhter validation of the PCR method in field conditions is needed to evaluate its performance.
- Maintaining good honey house hygiene and good beekeeping practices are the most important measures to control SHB where eradication is no longer the objective, taking into account that no approved veterinary medicine is available in the EU.
- Traps could be used to reduce the SHB population in infested areas where eradication is no longer the objective, based on experiences in Australia, Canada and the USA.
- No specific control measures are available to keep honey bee queen production free from SHB in an infested area where eradication is no longer the objective.
- There is no EU legislation in place regarding movement control of honey bees, bumblebees or commodities within an SHB-infested area where eradication is no longer the objective.

TOR4: surveillance in assessing freedom of areas from SHB including the size (radius of) of the areas to be surveyed in order to provide solid bases for regionalisation policy

- According to modelling that took into account implementation of inspection and mitigation measures as done by Italy including a protection zone of 20 km, reducing the surveillance zone from 100 km to 50 km will at least double the probability of SHB escaping undetected from the surveillance zone, from 0.027 to 0.053.
- The OIE requirement to implement a 5-year monitoring to substantiate SHB freedom is based on the current knowledge of the biological characteristics of the pest. The 5-year period could be used until data become available as basis for a more detailed assessment.
- Passive surveillance is implemented in all MS as SHB cases are notifiable. Guidelines on surveillance strategies have been published by the EURL.

TOR5: susceptibility of kept bumblebees (*Bombus terrestris*) to SHB or their capability to spread SHB as vectors

- A field experiment showed natural infestation of commercial bumblebee *B. impatiens* colonies placed next to SHB-infested honey bee hives. However, no data on SHB infestation in natural bumblebee colonies have been published.
- Food resources and conditions in bumblebee colonies are attractive to SHB and suitable for its development. Therefore, bumblebee colonies acting as a reservoir for SHB cannot be excluded.

5. Recommendations

TOR 1: risk of survival, spread and establishment of SHB in and from Calabria and Sicily to other parts of Italy and the EU

- Perform detailed epidemiological studies on the Italian outbreak to improve knowledge on introduction, survival, spread and establishment of SHB in Europe.

TOR 2: risk-mitigating factors that could potentially be effective in ensuring safe intra-EU trade of live bees, apiculture products and by-products as regards the transmission of SHB

- The assessment assumed perfect implementation of visual inspection, although this might not always be the case in practice. Therefore, it is recommended that the SHB status of the area of origin of consignments be taken into consideration when issuing health certificates for intra-EU movement of bee consignments, as is already done in the case of import from third countries.
- Strengthening visual inspection, protection from infestation by the use of a fine mesh and issuing a health certificate for intra-EU trade of queen bees, within 24 hours before dispatch, could reduce the risk of SHB transmission via consignments.
- A register of the location of apiaries, ownership and number of hives within an apiary/area, together with tracking information on the travel route of shipments, is essential to facilitate epidemiological investigations in the event of an outbreak.
- Even in the absence of a national registration system, it is recommended that beekeepers keep records of bee movements to facilitate investigation of outbreaks.
- It is recommended that movement restrictions on the movements of honey bees, bumblebees and commodities from infested to non-infested areas be maintained until SHB is eradicated, to prevent spread of the pest.

TOR3: risk-mitigating factors and methods in apiaries, alternative to currently employed complete destruction of the apiary and additional risk-mitigating factors that may be applied in controlled environments for queen producing

- Strengthening good honey house hygiene standards and good beekeeping practices are the most important measures to keep SHB infestation at low level in an infested area where eradication is no longer the objective.
- Soil treatment with pyrethroids to control SHB should be applied only in case of comb damage in an area where eradication is no longer the objective.

TOR4: surveillance in assessing freedom of areas from SHB including the size (radius of) of the areas to be surveyed in order to provide solid bases for regionalisation policy

- Training of beekeepers and veterinary inspectors will facilitate early SHB detection.

TOR5: susceptibility of kept bumblebees (*Bombus terrestris*) to SHB or their capability to spread SHB as vectors

- Studies are needed on the capacity of *B. terrestris* occurring in Europe to act as SHB host.

- Kept bumblebee boxes should be destroyed after the pollination service.

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Glossary

Apiary	A beehive or group of beehives whose management allows them to be considered as a single epidemiological unit.
Beehive	A structure for the keeping of honey bee colonies that is being used for that purpose, including frameless hives, fixed frame hives and all designs of moveable frame hives (including nucleus hives), but not including packages or cages used to confine bees for the purpose of transport or isolation.
Colony	A community of bees having a queen and thousands of workers on combs; for part of the year may contain drones and brood.
Ownership network	Referring to a higher risk of an apiary being infested if the owner has another apiary that is infested. However, no statements can be made about the mechanisms underlying this higher risk
Package bees	From 1 to 2.5 kg of adult bees, with or without a queen, and usually accompanied by a can of sugar syrup, contained in a ventilated shipping case.
Robbing	Stealing of nectar, or honey, by bees from other colonies.
Queen excluder	A selective barrier, either a sheet of perforated metal or plastic or a wire grid in a frame, inside the beehive applied with the aim of limiting the queen's access to the honey supers.
Super	A box with frames in which bees store honey; usually placed above the brood nest.
Swarm	The aggregate of worker bees, drones and queen that leave the mother colony to establish a new colony or formed by the beekeeper (artificial). Neither the natural nor the artificial swarm (package bees) contains combs and brood.
Slumgum	The residue of the beeswax rendering process. When the beeswax from brood comb is rendered to produce clean wax, it leaves behind the pupal lining, wax moth cocoons, excrement from larvae, and other residual debris included in the original material.
Transport	A two-phase process of moving a consignment, starting with the preparation of the consignment and ending with arrival of the consignment at the place of destination.

Abbreviations

DFB	dried-fruit beetle
EURL	European Union Reference Laboratory of Honeybee Health
Gy	gray (a unit of measurement of absorbed radiation)
MRL	maximum residue limit
MS	Member State
NUTS	nomenclature of territorial units for statistics
LC_{50}	lethal concentration resulting in 50% mortality
OIE	World Organisation for Animal Health
PI	phytosanitary irradiation
RH	relative humidity
RMSE	root mean square error
SD	standard deviation

SHB	small hive beetle (*Aethina tumida*)
SIT	sterile insect technique
TOR	Term of Reference
TRACES	TRAde Control and Expert System

Appendix A – Calibration and validation of a simple empirical soil temperature model

Method, performance and limitations

For a spatial grid-based application, a simple soil temperature model for daily mean soil temperature at 20 cm soil depth was developed using statistical models. The assumption is that this model approach is less data and calculation intensive than other dynamic approaches but with comparable performance given the uncertainty in a number of variable spatial representative factors driving soil temperature (i.e. soil conditions, soil cover dynamics, etc.).

The linear multiple regression model is based on the daily mean air temperature at 2 m above ground (which is the World Meteorological Organization standard for air temperature measurements at weather stations) as driving factor for the daily mean soil temperature at 20 cm soil depth as depending factor, where the daily mean air temperatures of the actual and past 4 days are used as predictors.

The multiple regression model was calibrated for different surface conditions, representing the main agricultural land-use types: (1) permanent grassland and forest (permanent soil cover), (2) arable cropping (temporal dominating soil cover by crop canopies/mulch/crop residuals) and (3) orchard conditions (i.e. vineyards with partly permanent bare soil surface). After calibration using measured data obtained from various sites in Austria, the model was validated for grassland on different sites with similar soil surface conditions as in Austria.

The basic assumption of this approach is that soil surface conditions, together with air temperature (which is strongly driven by the surface energy balance), are better predictors of soil temperature than other influencing factors such as soil physical conditions. Further, the approach (if applied at grid scales) represents spatial homogeneity of soil surface cover conditions (i.e. canopy, degree of soil cover) as given in the calibration sites (Table 6). In addition, topographical characteristics, such as aspect and slope, that affect surface energy balance are neglected, which can modify soil temperatures strongly at smaller scales. Further, the approach does not consider strong temporal changes in soil surface cover characteristics such as change from crop to bare soil conditions after harvest and, in particular, temporary snow cover effects in winter (Figure 12). Any deviation of these basic assumptions from the calibration reference may lead to temporal deviations of the calculated from the real soil temperature.

The calibration and validation for 20 cm soil depth was carried out only at Austrian sites (Table 6) and should be seen as a provisional solution; as more data are collected, further calibration and validation could be performed. Independent datasets for validation of Equations 2 and 3 below are needed (ongoing preparation or search for appropriate data is necessary; see Table 7). Air and soil temperature measurements were not always taken at the same site, but are representative for air temperature. The statistical performances of the models are shown in Table 7 and are based on multiple years of measurement data, including during winter. As can be seen, the determination coefficient (R^2) of the model for soil temperature (mean air temperature on a particular day and on the previous 4 days) is around 0.91 for all three calibration sites and the square root mean square error (RMSE) is around 2°C. Both RMSE and standard deviation (SD) might be improved by excluding winter from the analysis (because of temporal disturbing snow cover effects).

Finally, Table 7 shows the model performance of the three equations in relation to the measured 20 cm soil temperature depths, where R^2 is between 0.91 and 0.97 and the SD lies between 1.42 and 2.46°C for the calibration and validation sites. The differences in the mean of the predicted versus estimated soil temperatures of the full periods are +0.6 and –0.4°C for the two validation sites (Table 7). This allows the application of temperature sum calculations with moderate biases.

In conclusion, further validation and/or re-calibration requires accurate soil temperature measurements to be obtained at different sites throughout Europe with similar land-use but with different climatic conditions. This is necessary to assess Europe-wide performance of the model or identify, for example, specific regional conditions.

The equations developed at the Austrian calibration sites

(related to agricultural land-use type of the calibration sites)

Equation 1: Valid for sites with permanent full soil cover and vegetation (i.e. permanent grassland, closed forest canopies, other permanent canopies). Eq. 1 was calibrated and validated under permanent grassland.

$$ST20_{(d)} = 2.35 + 0.186*AT_{(d)} + 0.181*AT_{(d-1)} + 0.115*AT_{(d-2)} + 0.052*AT_{(d-3)} + 0.256*AT_{(d-4)}$$

Equation 2: Valid for sites subject to standard crop rotation with annual crops and catch crops on cultivated (arable) soil (i.e. cereals, maize) with only temporary (not dominating) bare soils. Eq. 2 was calibrated on one site of Austrian standard crop rotation including cereals, maize.

$$ST30_{(d)} = 3.93 + 0.188*AT_{(d)} + 0.006*AT_{(d-1)} + 0.106*AT_{(d-2)} + (-0.110*AT_{(d-3)}) + 0.685*AT_{(d-4)}$$

Equation 3: Valid for partly covered soil or land-use with partly bare (uncovered) soil (i.e. orchards, olive, vineyards). Eq. 3 was calibrated only in Austrian vineyard.

$$ST20_{(d)} = 3.68 + 0.379*AT_{(d)} + 0.188*AT(d\_1) + 0.114*AT_{(d-2)} + 0.108*AT_{(d-3)}) + 0.252*AT_{(d-4)}$$

where ST20 = daily mean soil temperature in 20 cm soil depth in °C; ST30 = daily mean soil temperature in 30 cm soil depth in °C; AT= daily mean air temperature 2 m above ground in °C; and d = actual day, d–1 = 1 day before, etc.

Table 6: Characteristics of the measurement sites used for model calibration and validation

	Surface condition	Soil	Topography	Distance air/soil temp. measurement	Site name (of soil temperature measurement)
Calibration site characteristics					
Grassland type (Eq. 1)	Meadow (2–3 cuts)	Loamy silt	Flat	100 m	Obersiebenbrunn/Lower Austria
Crop type (Eq. 2)	Crop rotation incl. cash crops (bare soil periods)	Sandy chernozem	Flat/lysimeter	5 km	Pucking/Upper Austria
Orchard type (Eq. 3)	Vineyard	Silty chernozem	Gently rolling terrain (almost flat)	25 km	Purbach
Validation site characteristics					
Grassland type (Eq. 1)	Meadow (3–4 cuts)	Loamy sand	Gently southern slope	7 km	Kirchberg/Walde
Grassland type (Eq. 1)	Meadow (3–4 cuts)	Loamy silt (wet site)	Flat/lysimeter	10m	Pettenbach
Crop type (Eq. 2)	–	–	–	–	–
Orchard type (Eq. 3)	–	–	–	–	–

Table 7: Provisional statistical performances of model calibration and validation for the three land-use types based on multiple years, including winter season for daily mean soil temperatures. Predictors are the driving air temperatures

Calibration results

	R^2	RMSE (°C)	R	SD (°C)	Calibration period	Soil depth (cm)	Comment
	of predictors		Model performance				
Grassland type (Eq. 1)	0.91	1.95	0.95	1.87	01/1983–12/2000	20	
Crop rotation type (Eq. 2)	0.89	2.01	0.94	1.88	09/1996–02/1998 (with gaps)	20	Further 20 cm datasets in preparation
Orchard type (Eq. 3)	0.93	2.04	0.96	1.97	07/1999–05/2002 (with gaps)	20	Search for further 20 cm datasets

Validation results

	–	R	SD (°C)	Mean bias (°C)[(a)]	Validation period		
		Model performance					
Grassland type (Eq. 1)	–	0.97	1.42	0.60	05/2003–05/2008 (with gaps)	20	Kirchberg/ Walde
Grassland type (Eq. 1)	–	0.91	2.46	–0.42	01/1995–09/1999	20	Pettenbach
Crop rotation type (Eq. 2)	–	–	–	–	–	–	20 cm datasets in preparation
Orchard type (Eq. 3)	–	–	–	–	–	–	Search for 20 cm datasets

(a): Predicted minus measured.

The following graphs demonstrate examples of the specific performances of the models during the calibration and validation periods for predicting the daily mean soil temperatures. Figure 12 demonstrates the effect of snow cover on the model performance. During winter 1992/1993, no significant snow cover was existent, but in winter 1993/1994 snow cover led to a deviation of simulated temperatures (in fact, the simulations represent soil temperatures without snow cover only).

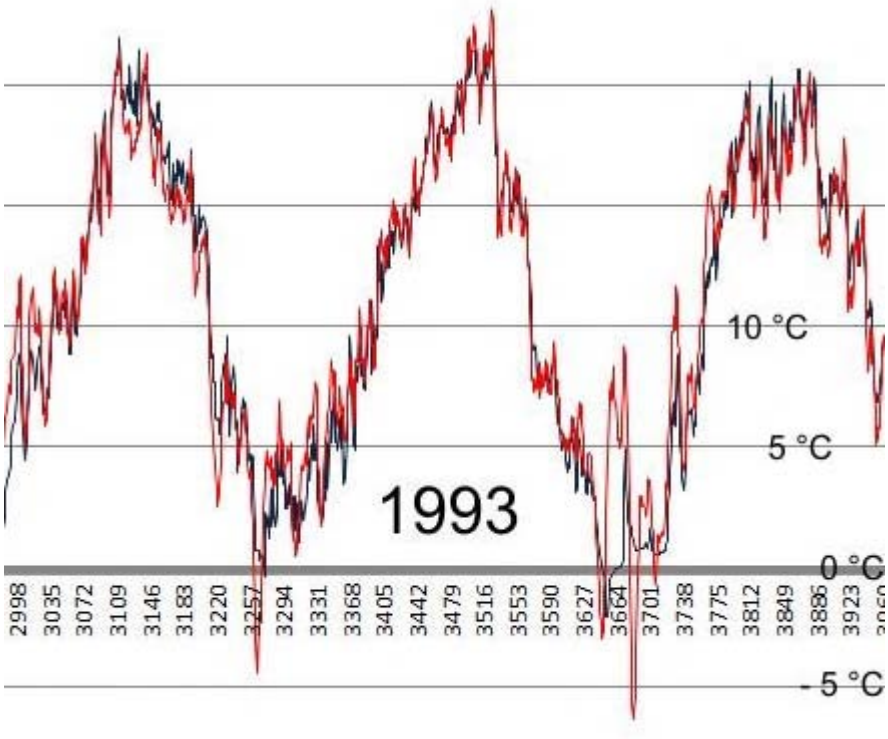


Figure 12: Simulated (red line) vs. measured (black line) daily mean soil temperatures at 20 cm soil depth at the calibration site (grassland) Obersiebenbrunn

Figure 13 shows the performance of the grassland model (Eq. 1) at the validation site Kirchberg. This site is characterised by regular snow cover during winter. In this case the simulated soil temperatures deviate regularly and significantly during winter.

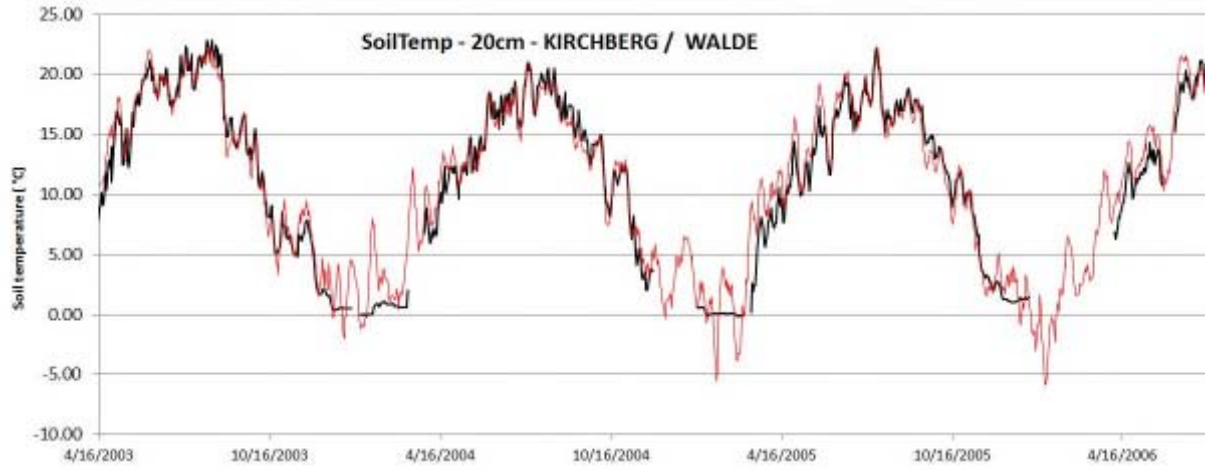


Figure 13: Simulated (red line) and measured (black line) daily mean soil temperatures at 20 cm depth at a grassland validation site in Kircherg/Walde. Gaps in the black line mean that no measured data are available

Figure 14 presents the results of the calibration site Purbach for vineyard conditions. Here again the snow cover effect, resulting in deviations during winter, is partially significant in the period considered. The blue dotted line shows the simulated temperature for that site using Eq. 1 for grassland conditions; lower temperatures during the summer period are simulated owing to the damping effect of soil cover on the soil surface temperature.

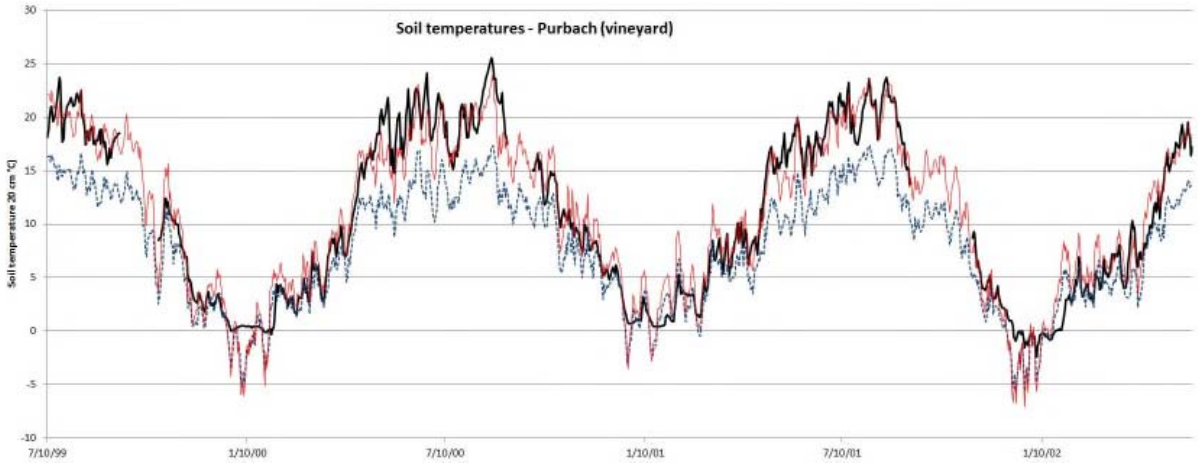


Figure 14: Simulated (red line) and measured (black line) daily mean soil temperatures at 20 cm depth at the vineyard calibration site in Purbach. The blue dotted line shows the simulation for grassland conditions for that site

Appendix B – SHB spread models

Mathematical modelling of the SHB spread

Here, the details of the mathematical models used to investigate the spread of SHB in Italy and the outbreak and demographic data used in the modelling are presented.

Outbreak data

The SHB outbreak is described in Section 3.1.1. Location (latitude and longitude), dates and outcome (SHB detected or not) for inspected hives were obtained from the Italian authorities. Only inspections carried out up to 30 September 2015 were included in the analysis (Figure 15).

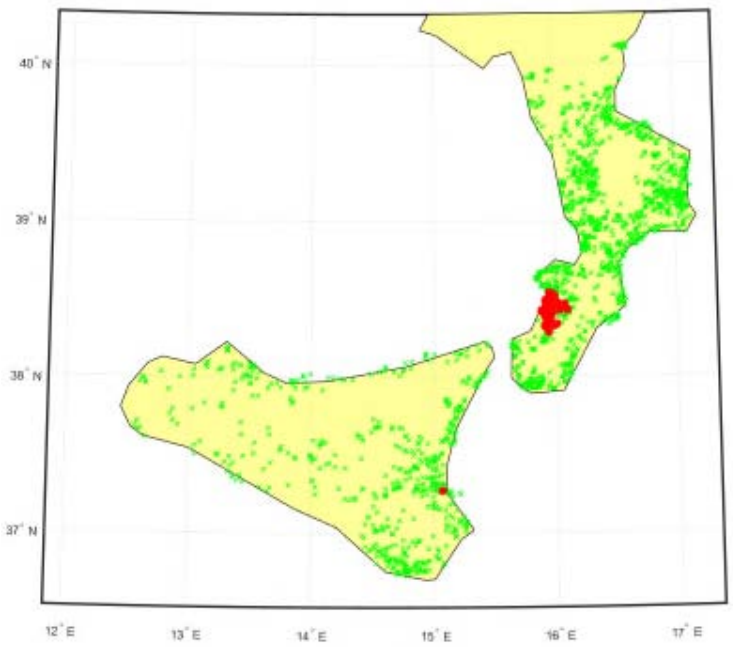


Figure 15: Inspection data from Calabria and Sicily showing the location of SHB-negative (green crosses) and SHB-positive sites (red circles)

Demographic data

The locations of all registered apiaries (totalling 3 888) in six regions of southern Italy—Molise, Campania, Apulia, Basilicata, Calabria and Sicily (Figures 1 and 16)—were obtained from the Italian authorities. These data were assumed to be incomplete, as registration with the veterinary service is mandatory but the comprehensive national database has not yet been completely populated. However, the available data were sufficiently detailed to estimate apiary density and to carry out the analysis. The dataset of all registered apiaries was combined with the negative and positive inspection data to form a comprehensive dataset of all registered apiaries in the south of Italy. Merging the two datasets involved putting together multiple visits to the same apiary, and removing duplicate apiaries present in both the inspection and registration data (i.e. with the same longitude, latitude and owner); a total of 6 540 individual apiaries were found to be present in the merged dataset. Here we define individual owners by the eight-digit identifier code.

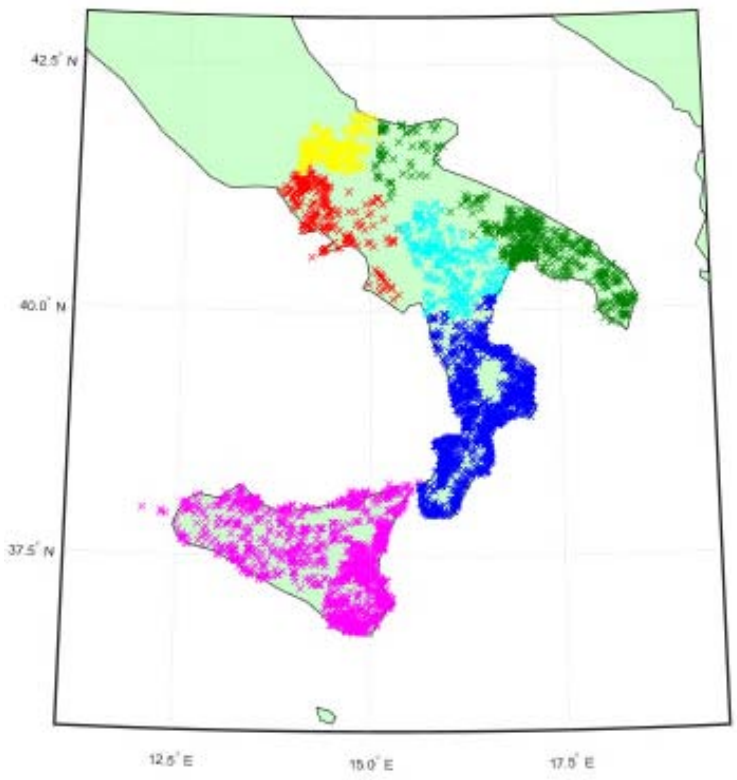


Figure 16: Map of southern Italy, with known apiary locations. Colours and total numbers of apiaries are as follows: Campania (red, 293), Molise (yellow, 329), Apulia (green, 621), Basilicata (cyan, 321), Calabria (blue, 2 889), and Sicily (magenta, 2 087).

In the case of the remaining 15 NUTS2 regions of (mid and northern) Italy, estimates for the numbers of apiaries in each region were available to the working group, but not their locations or ownership details[42]. The regional-level data were used to generate synthetic apiary-level data by generating a location for each farm in a region by sampling a point uniformly at random from within the boundary of that region. The synthetic datasets were generated using the maptools (Bivand and Lewin-Koh 2015) and spatstat (Baddeley and Turner, 2015) packages in R (R Core Team, 2015). The spread of SHB was modelled through these 'artificial' apiary networks, as a proxy for the rate of spread through the whole of Italy.

Modelling approach

Taking the geographical locations and ownership details of all apiaries in Calabria, two similar mathematical models were constructed allowing transmission between apiaries. For this, an SIR (Susceptible–Infested–Removed) model was used, similar to that used for AFB (Datta et al., 2013).

Spread of SHB from an infested apiary to a non-infested one is modelled using a rate function *R*; this is where the two models differ. In the first case, only distance is considered for transmission (henceforth referred to as the 'distance-only' model), with a distance-dependent kernel which decreases with increasing distance between apiaries, whereas the latter case (henceforth referred to as the 'distance and ownership' model) incorporates both distance and ownership in its construction. A limit of 30 km is imposed on the transmission of SHB from one apiary to another, based on scientific literature and expert opinion indicating maximum travelling distances of 5–15 km.

Parameter estimation

The parameters in the model were estimated in a Bayesian framework. Several transmission parameters were estimated, along with the probability of inspectors detecting SHB at an infested

[42] Data from the new Italian database became available only at the end of the mandate, when it was too late to rerun the models.

apiary. Also infestation times were estimated for SHB-positive apiaries in the data, along with possible unknown 'occult' infestations, which are not present in the data but likely to have occurred given the dynamics of SHB dispersal. A Markov chain Monte Carlo (MCMC) likelihood scheme was used to generate samples from the joint posterior density of the parameters (see Datta et al., 2013).

Simulating SHB outbreaks

The results from the MCMC scheme are fed into a stochastic SIR model, designed to recreate the outbreak thus far, as well as simulating forwards in time. In short, the apiary network is set up as in the MCMC scheme, and a single infestation dropped in to begin the outbreak. In the case of comparing with the Calabria dataset, the earliest infestation in the dataset is set off on the first day of simulations (1 June 2015). The outbreak is allowed to run, with visits occurring as in the data. Infestations are removed from apiaries if SHB is detected at a visit.

Firstly, a within-Calabria model is run, using outputs from both of the models, to predict the probable state of the outbreak up to 24 June 2015 (i.e. whether SHB is likely to be present in apiaries, or whether the inspection effort was sufficient to wipe it out). Following this the time taken to reach three locations was investigated: (i) the northern border of Calabria, (ii) the border between Molise and Abruzzo, where the furthest north registered apiary locations in the dataset are and (iii) the whole of Italy, until reaching the furthest northern apiaries at the border with other European countries (being Austria, France, Germany, Liechtenstein and Switzerland – the time looked to reach Liechtenstein and Austria was analysed). As no data were available in time to the working group on the ownership network for most regions of Italy, only the distance-only model was used to simulate the spread of SHB through the whole of Italy.

Results

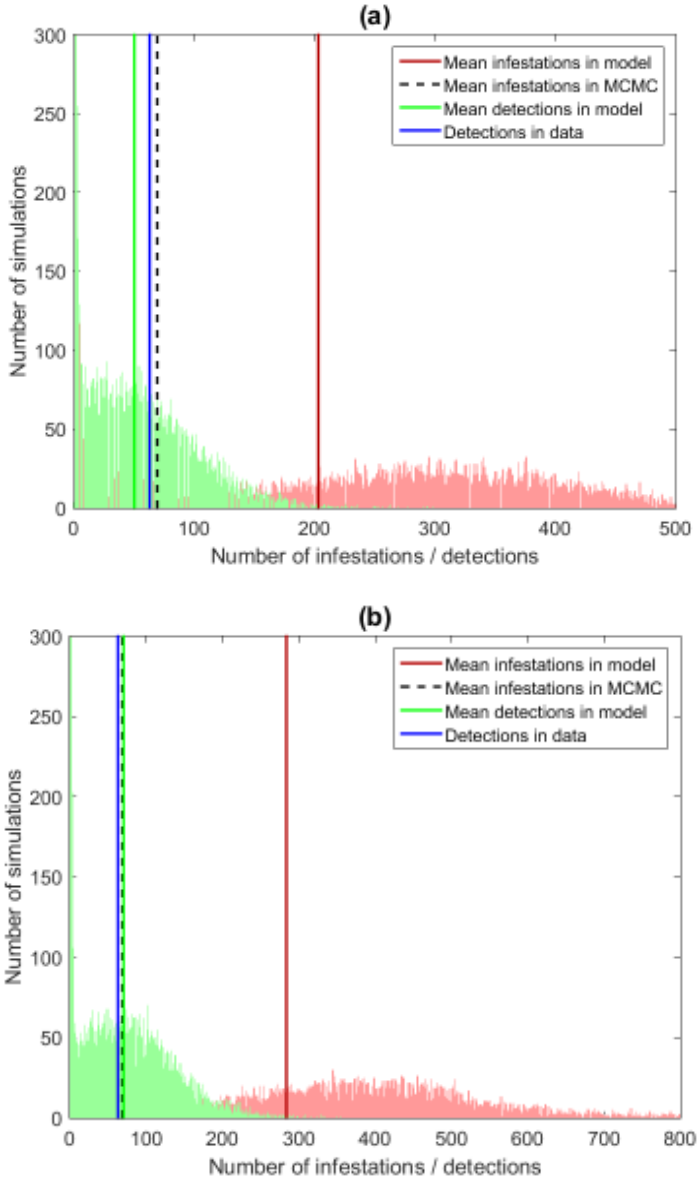
Model fitting

The MCMC is well mixed, and quickly reaches a region of parameter space where the likelihood (i.e. the similarity between model and data) is high; all parameters move in stable and predictable fashions around peak likely values. All parameter constants form Gaussian-shaped histograms for both the distance-only and distance and ownership models, indicating that the system has reached a favourable region of parameter space (Results not shown).

The parameter distributions for the distance and ownership model imply that, although most transmission events occur by distance-related dispersal, ownership is a major avenue of infestation. This is likely due to the large number of SHB-positive apiaries with the same owner; out of 63 confirmed cases in Calabria, 36 had the same owner as another infested apiary. Hence, owner movements between infested apiaries are an important factor in onward transmission of SHB. The distance kernel for both models suggests that distance-based transmission occurs only over short distances; an apiary immediately adjacent to an infested one is twice as likely to be infested as one roughly 2.5 km away from the same apiary, which makes distance transmission concentrated within an area. This is corroborated by the positive inspection data, as within Calabria infested apiaries are at most 28 km away from each other. Finally, from the analysis inspectors are very likely (more than 95%) to spot the beetle if it is present in a colony.

Stochastic simulations

The results of running 10 000 simulations in Calabria from the earliest assumed infestation date until the final inspection (i.e. 1 June 2014 to 30 September 2015), using values sampled from the MCMC, are shown in Figure 17, and compared with values from the data and MCMC. In general figures are reported for the 'distance only' model, and 'distance and ownership' results in parentheses afterwards.



After inputting sets of parameter constants randomly chosen from the MCMC, the SHB outbreak is set off at the primary detection location on 1 June 2014, and then allowed to spread until the date of last inspection (30 September 2015). Inspections are carried out according to the data, and SHB removed when it is detected. The sizes of the outbreaks (i.e. total number of infestations, including removed ones) are shown by the red histogram (mean shown by solid red line) and numbers of detections are shown by the green histogram (mean shown by solid green line), with the number of simulations producing these numbers on the y-axis. To show the closeness of fit to the data, plotted are the total number of detections in the data (63, solid blue line) and the average number of infestations over 200 000 iterations of the MCMC (69 in both models, dashed black line), which is detections in the data plus an average of 6 occult infestations.

Figure 17: Results of 10 000 stochastic SIR simulations, using values sampled from the MCMC scheme, for (a) 'distance only' and (b) 'distance and ownership' models

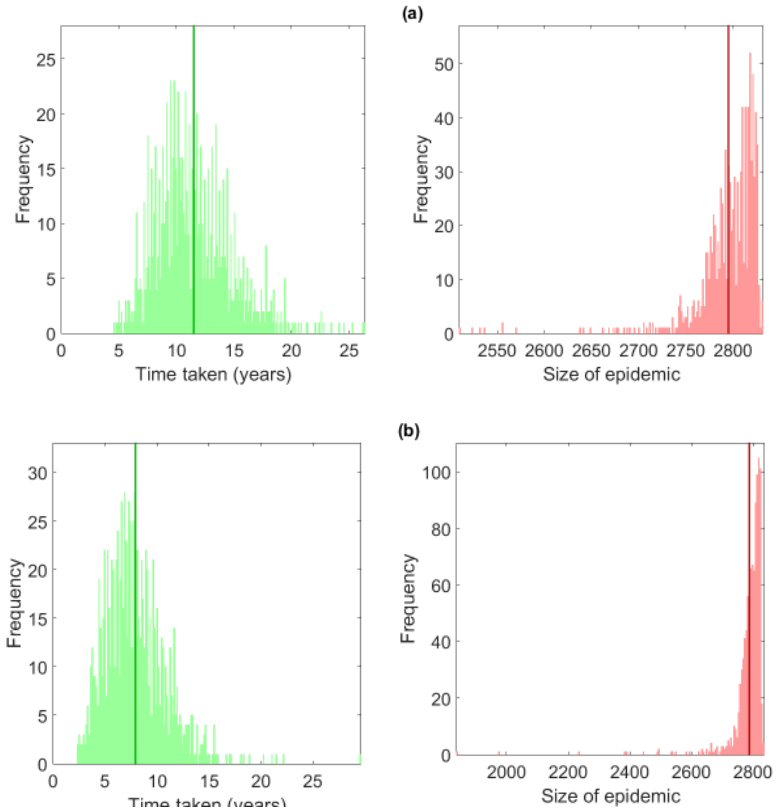
The MCMC does not predict a high number of occult infestations, and the match between detections and predicted infestations is very high (hence, the blue and black dashed lines in Figure 17 are close together). The number of detections is relatively close to 59, with a mean of 50 (70); however, this varies highly over the 10,000 simulations, with a maximum of 299 (356) detections. The majority of outbreaks are larger than the 'reported' outbreak of 63 in Calabria (69.4% in the distance-only model, 73.8% in the distance and ownership model), with a mean outbreak size of 203 (284) and a maximum outbreak size of 749 (1 569). Outbreaks tend to be higher in the 'distance and ownership' model than in the 'distance only'' model; this is probably because the beetle can jump large distances via ownership links, spreading to nearby apiaries in previously non-infested areas, before repeating the process. The mean outbreak size in simulations is much higher than the 69 predicted by the

MCMC. This is generally expected in these simulations, owing to both stochasticity in transmission and to the large number of apiaries in the dataset which are not visited by inspectors, and hence, if infested, will remain so for the rest of the simulation, as well as transmit onwards.

From an eradication standpoint, over the 10 000 simulations the infestation dies out 18.7% (23.3%) of the time; in other words, occasionally in a simulation the inspections carried out in September 2014 to June 2015 are sufficient to remove all the SHB present in apiaries, and none is left at the end of the simulation, but more often than not SHB remains at the end of the simulation. From a management standpoint, the interpretation of this is that control of the pest is difficult but not impossible, and extinction is a possibility with a large and concerted inspection effort to wipe out the beetle and limit its spread.

For the remaining forward simulations, to start the epidemic we use the infestation statuses of apiaries following the 10,000 Calabria simulations (see Figure 17), picking only from simulations where SHB has not yet been eradicated.

Simulating the time required to reach the northern border of Calabria with the two models is shown in Figure 18.

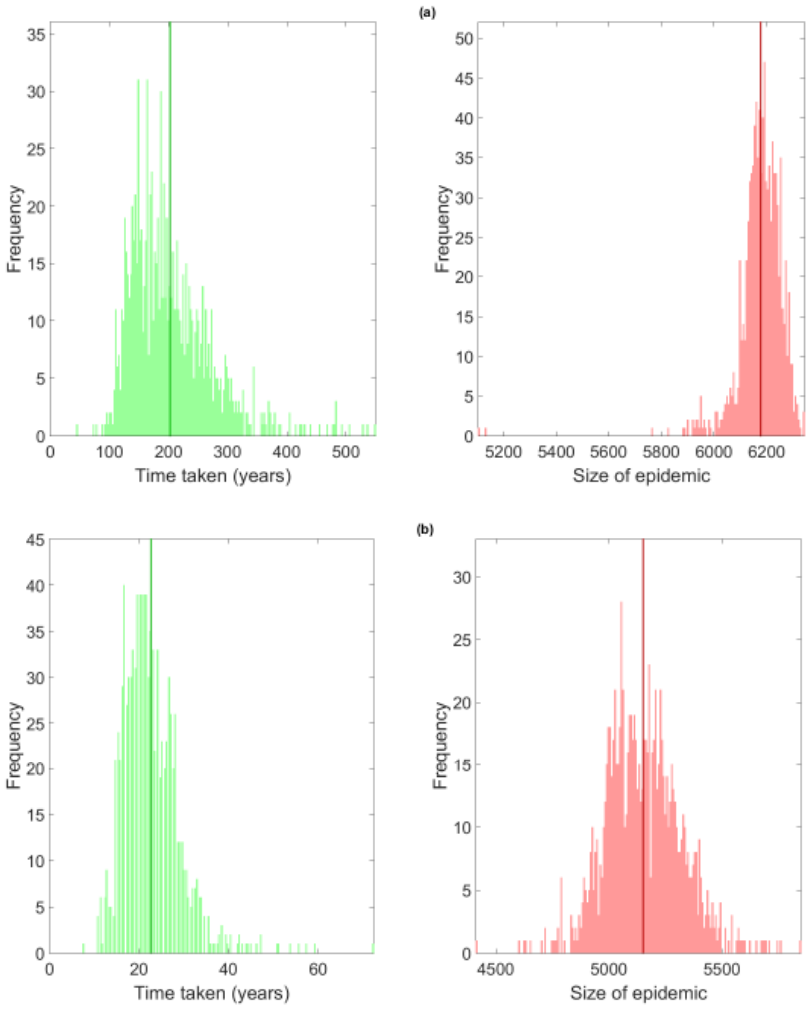


By 'northern border' we assume that at least one of the 50 most northern apiaries in Calabria has become infested.

Figure 18: Predicted time for SHB to reach the northern border of Calabria (along with size of the outbreak at this point) based on 1 000 simulations, given that current inspection efforts have not wiped out SHB, for the (a) 'distance-only' and (b) 'distance and ownership' models

In the two scenarios, the average time to reach the northern border of Calabria is (a) 11.5 and (b) 7.91 years (Figure 18). The lower figure for the distance and ownership model implies that ownership links enable SHB to 'jump' across the landscape more rapidly, as distance is not taken into account with ownership links between apiaries—any infested apiary is equally likely to infest any other owned by the same beekeeper. The mean outbreak size by the time the border is reached tends to be most of the apiaries in Calabria (both means lie around 2 790, and the total number of apiaries modelled in Calabria is 2 889). Hence, in the majority of cases SHB has saturated the landscape to be able to move northwards.

The ownership network is known not only for Calabria, but for the six southern regions (i.e. Molise, Apulia, Basilicata, Campania, Calabria and Sicily). Hence we can calculate the time taken to reach the north of the known apiary network using both distance-only and distance and ownership models. The results are shown in Figure 19.



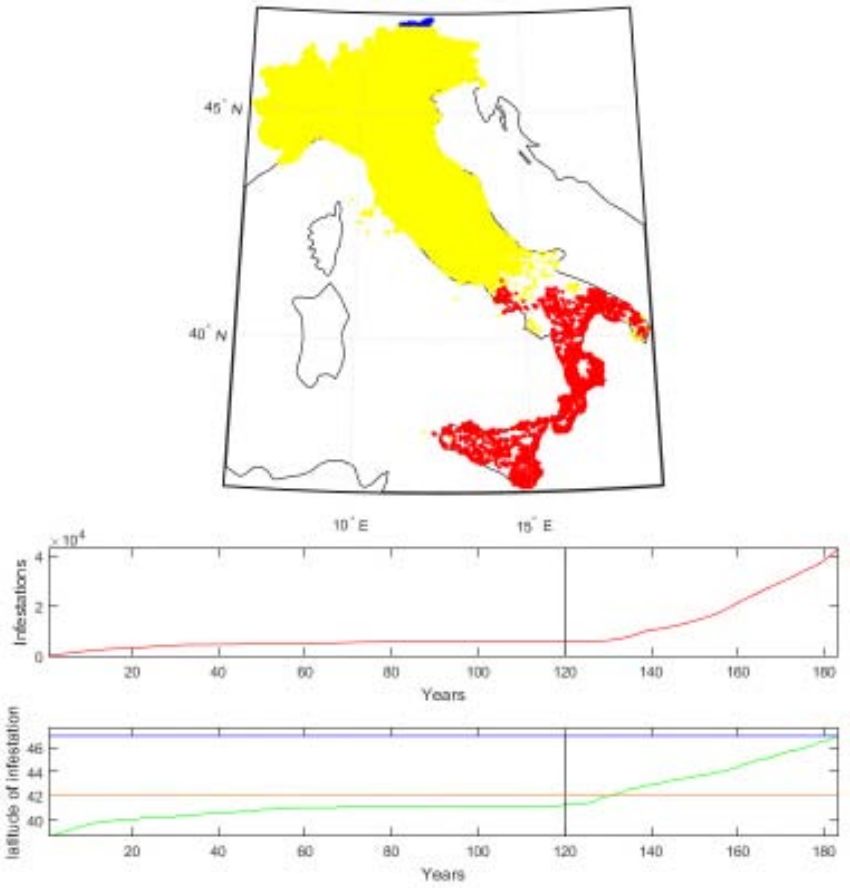
By 'northern border' we assume that at least one of the 100 furthest north apiaries in Molise (the furthest north region with registered apiary locations among those considered in this document) has been infested.

Figure 19: Predicted time for SHB to reach the northern border of the known apiary network (along with size of outbreak at this point) based on 1 000 simulations, given that current inspection efforts have not wiped out SHB: (a) 'distance-only' model, (b) 'distance and ownership' model

Here the difference in the time taken between the two models becomes much clearer; as the 'distance and ownership' model allows SHB to spread via ownership, the time taken is much lower than for model 1 (an average of 22.7 years compared with 202 years, almost ten times faster). The sizes of outbreaks at the end are slightly smaller for the 'distance and ownership' model (a mean of 5 151 compared to 6 178 out of a total of 6 540 apiaries). This demonstrates the importance of ownership to the beetle spreading, and highlights a major way in which the outbreak can be slowed down. If careful beekeeping practices can stop SHB from moving with the beekeeper between apiaries, then a major route of transmission can be reduced, increasing the probability of stopping the outbreak before it spread to other regions.

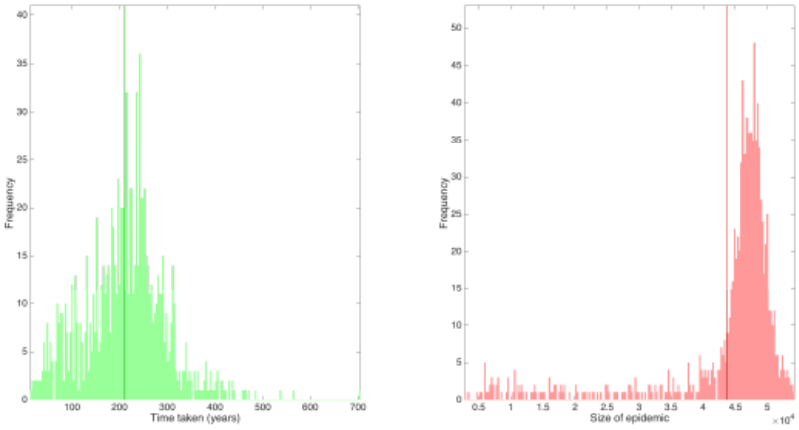
Simulating countrywide outbreaks in Italy is computationally intensive, and takes much longer than regional simulations. SHB is allowed to spread until one of the 200 most northern apiaries in the country are infested (these apiaries border Liechtenstein and Austria). The distance-only model is the

only model used, as ownership details are not known for the regions north of Molise. The results of one such simulation is given in Figure 20, and of 100 simulations in Figure 21.



Top plot: A snapshot 120 years into a countrywide outbreak. Apiary locations are in yellow, infested apiaries are in red, and the 'indication' apiaries for the outbreak having reached the northern border of Italy are in the north (blue). Middle plot: number of infestations over time. Bottom plot: latitude of the most northern infestation over time. Orange horizontal line gives furthest north apiary of known location (in Molise); blue horizontal line gives the latitude of the furthest south 'indication' apiary. Black vertical line in bottom two plots shows the point in the outbreak where the snapshot in the top plot is taken.

Figure 20: Output from a countrywide simulation of an SHB outbreak



Shown are the distributions of the number of years taken to reach the northern border, as well as the number of infestations at this point.

Figure 21: Summary of 100 simulations carried out over the whole of Italy.

The simulations in Figures 20 and 21 represent SHB spread through randomly placed apiaries in the remainder of Italy (apart from the six southern regions) and, as such, the results should be interpreted as only an estimate for the dynamics of a countrywide outbreak. Although outbreaks generally take a very long time to reach the northern border of Italy (mean of 240 years (Figure 21, left plot); in the example in Figure 20, it took 183 years), it is interesting to note that the majority of spread occurs once the northern regions are reached, where location data are unavailable and simulated apiary locations are used. The top plot of Figure 20 shows the status of apiaries 120 years into the outbreak, when the infestation has not yet reached the northern regions where apiaries are placed randomly. Apiary density is much higher in the north, and hence spatial spread will be much. This scenario is shown in the bottom plot in Figure 20; after crossing the most northern-most apiary in Molise (at around 137 years), where actual apiary locations are used, the pest moves swiftly further north, and the number of infestations rises dramatically. In the 137 years before reaching this tipping point, only 5 365 apiaries are infested, but in the 55 years after, another 42 085 apiaries are infested and the northern border of Italy is reached. This is important, because, if the apiary density in the southern regions is much higher than is given by the available data (see Figure 16), then the wavefront of the outbreak will move much faster than predicted here.

The size of outbreaks tends to be very high when the northern border of Italy is reached; out of a possible 56 080 apiaries, approximately 43 635 are infested on average (Figure 21, right plot). This is around 78% of all apiaries (excluding Sardinia), and hence the pest must be considered endemic long before the northern border is reached.

It is important to note that it is assumed that spatial transmission is the only method by SHB travel across the landscape. Because the necessary data were not available to the working group, the ownership network could not be used when simulating spread of the pest past the northern border of Molise, and transhumance and trade are assumed not to occur. If SHB has already crossed the border from Calabria into a region without movement restrictions, the pest can move more rapidly through colony and equipment movements than simply by flying between colonies.

If it were possible for data on honey bee colony movements (largely for seasonal pollination services) to be recorded, this information could potentially be included in the transmission models to investigate the added impact this has on SHB transmission (as a result of accidental beetle transfer between geographical locations). This would increase the reliability of the models in simulating the outbreak in Italy, as well as giving more accurate estimates of epidemic sizes and the time taken to spread across the landscape.

Appendix C – Data on bee consignments

Table 8: Number of live bees consignments exported from Italy to other European countries (source: TRACES)

Sum of Count consignments	Column Labels					
Row Labels	2010	2011	2012	2013	2014	Grand Total
AT	3	1	1	3	3	11
BE				4	3	7
BG					1	1
CH	5	4	7	18	7	41
CY				1		1
DE	1	1		1	12	15
DK	1	1	2	1	3	8
EE				3	3	6
ES			1			1
FI	4		2	4	15	25
FR	5	13	14	19	37	88
GB	3	5	1	6	11	26
LV				1	1	2
MT					1	1
RO	3	5	8	5	2	23
SI					2	2
Grand Total	**25**	**30**	**36**	**66**	**101**	**258**

Table 9: Number of live bees exported from Italy to other European countries (source: TRACES)

Sum of Total Animal Nbr	Column Labels					
Row Labels	2010	2011	2012	2013	2014	Grand Total
AT	55	20	20	66	140	301
BE				746	608	1354
BG					200	200
CH	1750056	2800147	431	4000597	3000430	11551661
CY				18		18
DE	5	35		80	964	1084
DK	200	175	350	175	375	1275
EE				775	1002	1777
ES			20			20
FI	1200		60	630	4536	6426
FR	10308	21686	1813	3410	51314810	51352027
GB	300	275	50	1500	1387	3512
LV				672	448	1120
MT					96	96
RO	319	675	1523	907	1100	4524
SI					8	8
Grand Total	**1762443**	**2823013**	**4267**	**4009576**	**54326104**	**62925403**

Appendix D – Estimation of the likelihood of introduction

A flow chart indicating the procedure to follow to estimate the likelihood of introduction based on binomial principles is shown in Figure 22. More details on how to estimate the likelihood of introduction can be found in EFSA (2012). The process of introducing a specific agent in an agent-free area is similar for different agents, and Figure 22 shows how this process could be applied to the particular case of SHB, with the EU or Italy as an agent-free area. SHB is assumed to be introduced via live bees, bee products to be used in apiculture or used beekeeping material from different areas where the agent is present. According to the legislation in place, live bees or bee products can be imported from territories which, at the date of export, are free from SHB. Thus, introduction of SHB through importation might occur if a recent infection has occurred in such territories without being detected before exportation. As, by definition, it is not possible to know, at the point of export, whether or not this event has occurred, it is necessary to estimate the probability that a sample (live bees and/or bee products) is infested in the population of concern in those areas (i.e. $\mathbf{P(D+)} = \rho$, true prevalence of infested materials in the population of concern).

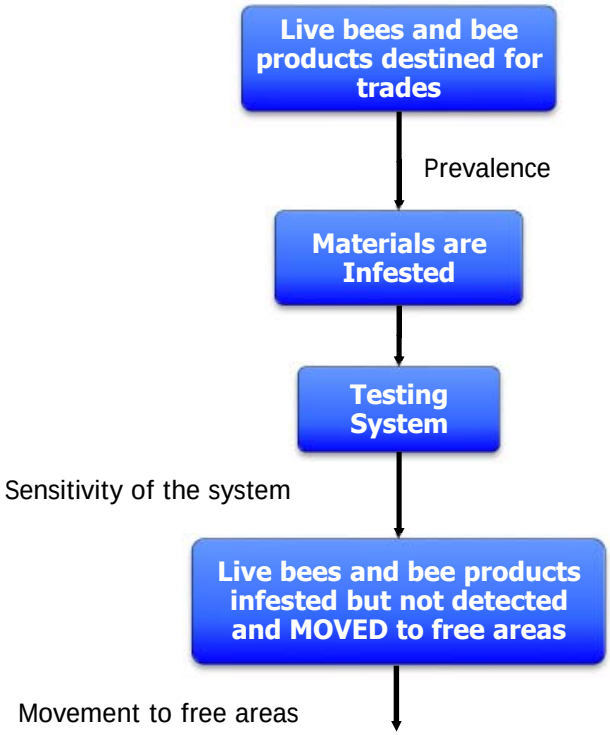


Figure 22: Procedure to estimate likelihood of introduction for SHB (adapted from EFSA, 2012)

In order to detect possible infested material (live bees and/or bee products), a testing system (in general based on a battery of tests, referred to as 'testing system' from here on) is usually set up. Therefore, the probability of detecting infested material will depend on the testing system characteristics, if such a system is in place. If material tests negative when it is in fact infested (i.e. a so-called 'false-negative', due to a lack of sensitivity of the testing system), the system fails and the disease is introduced. In contrast, if the testing system produces a positive result, regardless of the true status of the material, the material will not be accepted. Therefore, lack of specificity, leading to a 'false positive' (i.e. the material tests positive when it is actually non-infested) will not pose any risk of introduction of SHB.

Attention should be focused on estimating of the probability of obtaining a 'false-negative' result, i.e. the probability that material tests negative for SHB when it is actually infested. This probability can be estimated as follows:

$$P(D+ \mid T-) = \frac{P(D+) \times P(T- \mid D+)}{P(T-)}$$

This probability depends on the true prevalence ($P(D+)$. The remainder of the equation basically depends on the characteristics of the test, if one is used. The input information needed is, then, (1) the estimated prevalence of SHB in the population of concern and (2) the characteristics of the testing system. Hence, the probability that a single sample of material is SHB positive, given that the test result is negative, can be estimated using the following equation:

$$P(D+ \mid T-) = \frac{\hat{\rho} \times (1 - Se)}{(1 - \hat{\rho}) + \hat{\rho} \times (1 - Se)},$$

where $\hat{\rho}$ is the prevalence of SHB in the population of concern (estimate of the $P(D+)$) and **Se** is the sensitivity of the testing system.

Another important aspect to consider is that materials are not always moved individually. Usually, group of materials are moved (called shipment, determining the size of the import, N), and this influence the probability of introducing SHB into an SHB-free area via importation. In order to estimate the likelihood of introducing SHB into an SHB-free area in Europe, when more than one consignment is moved, the probability of at least one positive consignment escaping the testing system (and thus posing a risk of SHB introduction in a free area in Europe) must be estimated as follows:

$$P(x \geq 1) = 1 - \left(\frac{(1 - \hat{\rho})}{(1 - \hat{\rho}) + \hat{\rho} \times (1 - Se)}\right)^N$$

Ample details about the derivation of this formula are given in Sections 3.2 and 3.3 in EFSA (2012).

Appendix E – Ratings used to assess risk mitigation measures applicable to consignments

Table 10: Ratings of the effectiveness of risk mitigation measures

Name	Explanation
Negligible	The mitigation measures do not allow a reduction in the probability of survival, spread or establishment.
Low	The mitigation measures are unlikely to reduce the probability of survival, spread or establishment.
Moderate	The mitigation measures reduce the probability of survival, spread or establishment.
High	The mitigation measures eliminate the probability of survival, spread or establishment.
Unknown	The effects of the mitigation measures on survival, spread or establishment are mostly unknown.

Table 11: Ratings of the feasibility of risk reduction options

Name	Explanation
Negligible	The mitigation measures have many technical difficulties (e.g. changing or abandoning current practices, implementing new practices and/or measures) making their implementation in practice impossible.
Low	The mitigation measures can be implemented (e.g. changing or abandoning current practices, implementing new practices and/or measures) with technical difficulties.
Moderate	The mitigation measures can be implemented in practice (e.g. changing or abandoning current practices, implementing new practices and/or measures) with limited technical difficulties.
High	The mitigation measures are already in use in the risk assessment area or they can be easily implemented in practice.
Unknown	The feasibility of the mitigation measures is mostly unknown.

Table 12: Ratings used for describing the level of uncertainty

Name	Explanation
Low	No or limited information or data are lacking, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used.
Moderate	Some information or data are lacking, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.
High	The majority of information or data are lacking, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.

Appendix F – Production and trade of bumblebees

The current EU legislation concerning trade (Commission Regulation (EU) No 206/2010 for third country importations; Council Directive 92/65/EEC, amended by the Commission Decision 270/2010/UE for intra-EU trade) considers these units as 'environmentally isolated structures'. They shall be recognised establishments, and be supervised and controlled by the Competent Authority.

In the case of import of bumble bees (*Bombus* spp.) from third countries, the requirements of Commission Regulation (EU) No 206/2010 as amended by Commission Implementing Regulation No 1044/2013 should be certified:

a) the bumble bees (*Bombus* spp.) referred to in Part I of the certificate have been bred and kept under a controlled environment within a recognised establishment which is supervised and controlled by the competent authority;

b) the establishment referred to in Part I of the certificate was inspected immediately prior to dispatch and all bumble bees and breeding stock show no clinical signs or suspicion of disease including infestations affecting bees;

c) all colonies for import into the Union have undergone detailed examination to ensure that all bumble bees, brood stock and packaging do not contain the small hive beetle (*Aethina tumida*) or its eggs and larvae or other infestations, in particular *Tropilaelaps* spp., affecting bees.

The number of containers of bumble bees (*Bombus* spp.) should be declared, each containing a colony of a maximum of 200 adult bumble bees.

The packing material, containers, accompanying products and food are new and have not been in contact with diseased bees or brood combs, and all precautions have been taken to prevent contamination with agents causing diseases or infestations of bees.

In case of exchange (intra-EU trade), the requirements of Council Directive 92/65/EEC as amended by Commission Decision 2010/270/EU should be met:

a) The bees/bumble bees come from an area which is not subject of the prohibition order associated with an occurrence of American foulbrood [(the period of prohibition has been continued for at least 30 days following the last recorded case and the date of which all hives within a radius of three kilometres have been checked by the competent authority and all infected hives burned or treated and inspected to the satisfaction of the said competent authority);]

b) The bees/bumble bees come from an environmentally isolated structure recognised by and under the supervision of the competent authority of the Member State which is free of American foulbrood and was inspected immediately prior to dispatch and all bumble bees and breeding stock show no clinical signs or suspicion of the disease;]

c) The bees/bumble bees come from an area of at least 100 km radius which is not the subject of any restrictions associated with the suspicion or confirmed occurrence of the small hive beetle (*Aethina tumida*) or the Tropilaelaps mite (*Tropilaelaps* spp.) and where these infestations are absent;

The bees/bumble bees as well as their packaging have undergone a visual examination to detect the occurrence of the small hive beetle (*Aethina tumida*) or their eggs and larvae, or other infestations, in particular the *Tropilaelaps* mite (*Tropilaelaps* spp.), affecting bees.

Appendix G – Some SHB trapping systems

AJs Beetle Eater® trap (one of many trench trap designs) with non-crystalline Diatomaceous Earth (DE) (Mt Sylvia Absorbicide, http://www.mtsylviadiatomite.com.au/product) (Figure 23).

© Diana Leemon (photos provided to EFSA)

Figure 23: AJs Beetle Eater® trap full of dead SHB coated in diatomaceous earth. The dead SHB appear as small grey lumps

Vinyl mat with fibrous backing, when placed on top of frames in the top super, serves to prevent the build-up of burr comb as well as trapping SHB. A piece of vinyl is cut so that it can be folded over with the fibrous part facing in (bees can become trapped in the fibres as well). There should be a small area between the vinyl mat and the edges of the outer frames. SHB become entangled in the fibres when they seek refuge inside the folded vinyl mat (Figure 24).

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Figure 24: (A) Vinyl mat placement on the top of frames under the lid. (B) Vinyl mat removed from a hive and opened up to show dead trapped SHB. (C) Microscopic view to show how SHB become trapped when their legs get tangled in the fibrous backing of the vinyl mat

Chux® Superwipes® are cleaning cloths that can be folded and placed under the queen excluder on top of the brood box. Bees chew up the cloth and make it fibrous and SHB become trapped in the fibrous cloth when they seek refuge inside the folded cloth (Figure 25).

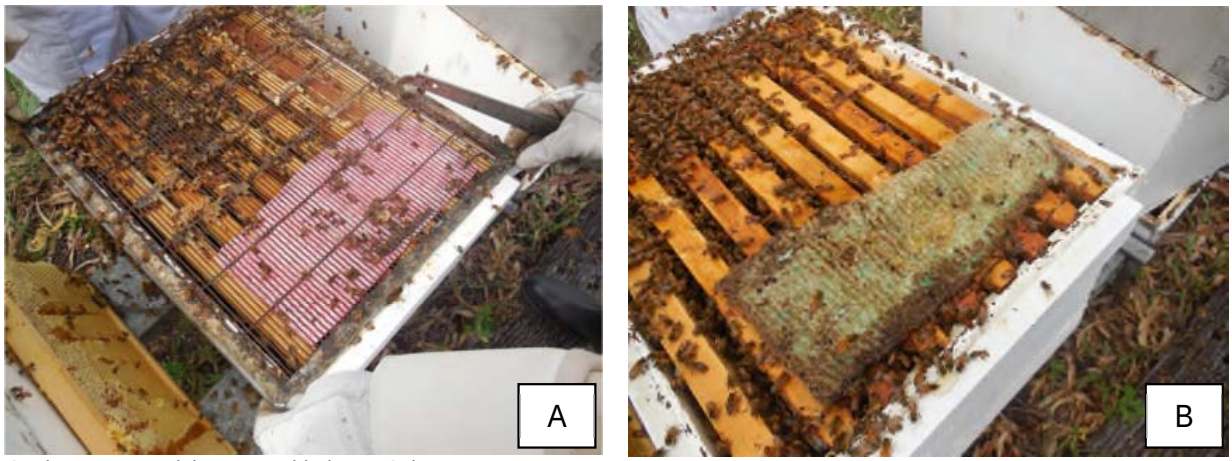

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Figure 25: (A) View of brood box with a folded Chux® cloth placed under the queen excluder. (B) Chux® cloth after a month in the hive, showing how bees alter the cloth, it becomes more fibrous and SHB become trapped. The bees can also seal the edges with propolis

A diagnostic strip, made of corrugated plastic, can be placed on the bottom board of a honey bee colony (see Section 3.4.4, Mechanical control). The strip is placed on the bottom board by easily sliding it through the flight entrance. It is left on the bottom board for at least 2 days to give SHB some time to find the shelter (Schäfer et al., 2008) (Figure 26). Examples of the Hood trap, West trap and CheckMite+ trap are provided in Figure 27.

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Figure 26: Diagnostic strip, made of corrugated plastic, placed on the bottom board of a honey bee colony (see Section 3.4.4, Mechanical control)

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Figure 27: (A) Hood trap, (B) West trap and (C) CheckMite+ trap